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# Effects of substituting different percentages of Azolla and duckweed as a partial substitute for soybean meal in feeding common carp fish (*Cyprinus carpio* L.)

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## ABSTRACT

The Azolla and duckweed and their mixture are one of the most important alternatives to feed in fish diets because they contain high levels of proteins and other nutrients and their role in improving production efficiency (2.5 and 5%, respectively), as well as their effects on the growth parameters of common carp during the experimental period (49 days). The experiment included feeding carp using seven experimental diets containing Azolla, duckweed and the mixture. The results of the statistical analysis indicated a significant superiority ($P \leq 0.05$) for the seventh treatment (the mixture 5%) for each of the characteristics of final weight, total increase, daily increase, the relative growth, and the specific growth rate compared to the control diet and the rest of the other treatments. A significant superiority of the same seventh treatment (5% mixture) was observed on the amount of feed intake and the food efficiency ratio compared to the control group and the rest of the treatments. The seventh treatment also recorded a significant increase in the rate of protein intake and the percentage of protein efficiency compared to the control and the rest of the treatments. The protein deposited rate and the protein produced value in the second treatment (Azolla 2.5%) were significantly superior ($P \leq 0.05$) in fish compared with the control and the rest of the treatments. It reached 7.25 g $fish^{-1}$ and 58.70% respectively. The results of the statistical analysis of the chemical composition of the fish body after being fed on different experimental diets in the proportion of protein showed that there were significant differences ($P \leq 0.05$) between the treatments, where the proportion of protein in the seventh treatment (the mixture 5%) increased significantly over the control and the rest of the treatments and amounted to 19.87%. There were no significant differences in the percentages of fat and ash among all treatments except for the control in the percentage of ash, which recorded a significant decrease compared to the rest of the treatments, amounted 1.89%. It is concluded that the mixture (Azolla and duckweed) can be used at a rate of 5% as a substitute for soybeans as the best growth criterion.

**Keywords:** *Azolla*, Soybean meal, duckweed, Ration, *Cyprinus carpio* L.
**Article type:** Research Article.

## INTRODUCTION

Most countries of the world are interested in providing animal products and securing them for the consumer to provide food security for their countries, including fisheries products, which are of high nutritional value because they provide a cheap source of animal protein (Hassan & Hashem 2016). Since it is an important source of mineral elements, vitamins, essential amino acids, and fatty acids (Mohanty *et al.* 2017), it has aided the global prosperity of the aquaculture sector, accounting for 51% of total production (Abdul Hamid 2019). The most important farmed fish is the common carp, *Cyprinus carpio* L., which is distinguished by its high production rates, rapid growth, resistance to difficult environmental conditions, ease of cultivation, and provision of its requirements. It is a model for fish to be farmed on a commercial scale (Gupta *et al.* 2005). To date, it represented 8% of the total global production of fish species, produced by fish farming projects (World Food Organization, FAO 2018). Fish feed is critical to the aquaculture industry's development (Dorothy *et al.* 2018). Azolla and duckweed are high-protein

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plants that can be used to substitute expensive protein sources like fishmeal and soybean meal in animal feed (Sajid *et al.* 2016).

**Nutritional value of duckweed and azolla plants**

The duckweed plant contains a crude protein of 15-43% on a dry matter basis, and it contains a high percentage of mineral elements such as potassium and phosphorous. It is rich in pigments, especially carotene and xanthophyll, as well as its abundance of production, as it doubles its live mass in a period of up to 2-3 days, and this is what made it enter as a material forage in fish feed rations (al-Tai 2010; Dorothy *et al*. 2018). The importance of the azolla plant stems from its high protein content, which ranges from 13 to 30% of its dry weight, as well as vital amino acids rich in lysine, as well as minerals like calcium, magnesium, potassium, iron, and vitamins like vitamin A and $B_{12}$ in feeding of common carp (Anitha *et al.* 2016; Das *et al.* 2018).

## MATERIALS AND METHODS

The study was conducted in the fish laboratory of the College of Agriculture and Forestry, University of Mosul, Iraq for a period of nine weeks including a two-week acclimatization period using glass basins of dimensions (40 × 60 × 40 cm) and equipped each tank with a Chinese-type (RS-510) air pump. The basins were filled with water stored in a large water tank inside the laboratory, and the water was left in it for a period of no less than 24 hours in order to ensure that it was free of chlorine, in addition to providing the experimental tanks with water at laboratory temperature and suitable to the temperature of the experimental tanks for fish, which is between 25 and 28 °C. It was done by air conditioners inside the laboratory. Fingerlings of common carp (*Cyprinus carpio* L.) were used; 98 fish, with an average weight of 18.71 ± 0.05, distributed among 14 glass tanks, with seven fish/pool and two replications/treatment. 25% of the aquarium water and the addition of pure water from the water tank in the laboratory, the tank water temperature was measured weekly and was at a rate of 24 2 °C (Crockford & Johnston 1990). The pH was measured weekly and its value was between 7.6 and 8.1, within the limits recommended by the Food and Agriculture Organization (FAO 1981).

**Composition of experimental rations components**

**Installation of experimental research:**

The primary feed materials were brought from the local markets to the fish laboratory and ground by a laboratory mill of German origin, and seven experimental rations were made by substituting azolla, duckweed and their mixture respectively, as a partial substitutes for soybean meal, in proportions of 0, 2.5% and 5% for the treatments ($T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$ and $T_7$) as shown in Table 1. The feeding system was based on providing three meals a day from the ration, and the amount of ration provided to the experimental fish was increased depending on the weight gain that occurred from the growth of fish from 3 to 5% of body weight. The fish were weighed every two weeks, using a sensitive electronic scale (0.00 g), a type of Chinese Citizen Origin: The experiment lasted seven weeks.

**Table 1.** Composition of experimental rations components (%) resulting from substituting different proportions of azolla, duckweed and their mixture as a partial substitute for soybean meal.

| **Experimental rations** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** | **$T_7$** |
|---|---|---|---|---|---|---|---|
| **Ingredients used** | **Control zero** | **Azolla (2.5%)** | **Azolla (5%)** | **duckweed (2.5%)** | **duckweed (5%)** | **Mixture (2.5%)** | **Mixture (5%)** |
| Fish meal | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| Soybean meal | 30 | 25.5 | 21.5 | 26.5 | 22.5 | 26 | 22 |
| Azolla* | --- | 4.5 | 8.5 | --- | --- | 2.25 | 4.25 |
| *Duckweed | --- | --- | --- | 3.5 | 7.5 | 1.75 | 3.75 |
| Barley | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Yellow corn | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 |
| Bran | 19 | 19 | 19 | 19 | 19 | 19 | 19 |
| Salt | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Vitamins | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Limestone | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Bonding article | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 2.** Chemical composition (%) of the experimental diets on the basis of dry weight with replacement ratios (2.5 and 5%) for each azolla, duckweed plants, and their combination.

| **Experimental rations Ingredients used** | **$T_1$ Control** | **$T_2$ Azolla (2.5%)** | **$T_3$ Azolla (5%)** | **$T_4$ Duckweed (2.5%)** | **$T_5$ Duckweed (5%)** | **$T_6$ Mixture (2.5%)** | **$T_7$ Mixture (5%)** |
|---|---|---|---|---|---|---|---|
| Raw protein | 25.60 | 25.88 | 26.74 | 26.81 | 26.55 | 26.91 | 26.94 |
| Raw fiber | 4.70 | 4.65 | 4.79 | 4.52 | 4.89 | 4.71 | 4.16 |
| Raw fat | 4.10 | 5.84 | 6.92 | 5.05 | 5.41 | 6.12 | 6.63 |
| Ash | 6.97 | 6.47 | 6.82 | 6.10 | 7.04 | 6.87 | 6.56 |
| Humidity | 7.20 | 6.56 | 7.11 | 6.84 | 7.02 | 6.82 | 7.22 |
| Nitrogen free extract (NFE) | 51.43 | 50.6 | 47.62 | 50.68 | 49.09 | 48.57 | 48.49 |
| *Metabolic energy (MJ $kg^{-1}$) | 13.28 | 13.8 | 13.91 | 13.72 | 13.57 | 13.81 | 13.97 |

*Metabolic energy was calculated based on Smith (1971) equation: ME (MJ $Kg^{-1}$) = Protein × 18.8 + Fat × 33.5 + NFE × 13.8.

**Methods of measuring the growth parameters of experimental fish**

The criteria for measuring fish growth were adopted to show the effects of substituting azolla and duckweed and their mixture as a partial substitute for soybean meal on its growth, represented by:

Total weight gain (g $fish^{-1}$) = Final weight (g) – Initial weight (g) (Pitcher & Hart 1982)

$$\text{Daily weight gain (g fish}^{-1}\text{)} = \frac{\text{Final weight - Initial weight}}{\text{Number of days}}$$

$$\text{Daily growth rate (g fish}^{-1}\text{ day}^{-1}\text{)} = \frac{\text{Weight gain (g)}}{\text{Duration of experiment (days)}}$$

$$\text{Relative growth rate (\%)} \frac{\text{Final weight (g) - Initial weight (g)}}{\text{Initial weight (g fish}^{-1}\text{)}} \text{(Uten, 1978)}$$

$$\text{Specific growth rate} = \frac{\text{Log final weight (g) – Log initial weight (g)}}{\text{Duration of experiment (days)}} \text{ (Jobling \& Koskela 1996)}$$

$$\text{Feed conversion ratio} = \frac{\text{Feed consumption (g)}}{\text{Total weight gain (g)}} \text{ (Uten 1978)}$$

$$\text{Feed efficiency ratio (\%)} = \frac{\text{Total weight gain (g)}}{\text{Feed consumption (g)}} \times 100$$

$$\text{Protein efficiency ratio} \frac{\text{Total weight gain (g)}}{\text{Protein intake (g)}} \text{ (Gerking 1971)}$$

$$\text{Protein productive value (\%)} - \frac{\text{Protein gain (g)}}{\text{Protein intake (g)}}$$

Protein intake = feed intake (g) × % crude protein (Jobling and Koskela 1996)

Protein gain (g) = Body protein final of experiment (g) - Body protein initial of experiment (g)*

Precipitated protein = % The crude protein in the fish's body for final weight - % The crude protein in the fish's body for the initial weight

**Chemical analyzes of fish growth experiment**

The estimation of the components (protein, fat, ash, moisture and nitrogen-free extract) present in the dry matter of the edible part of the fish body was carried out on the basis of dry weight based on the approved standard methods AOAC (2000) in estimating each of the protein, fat, ash and moisture, and calculating the free extract nitrogen is mathematically by the difference method, as shown in Wee & Shu (1989) and as follows:
Dissolved carbohydrates = 100 – [crude protein (%) + fat (%) + ash (%) + fiber (%) + moisture (%)].

**Statistical analysis**

Data analysis was performed statistically using complete randomized design (CRD) by statistical package for social science (SPSS, 2001) in analyzing the effect of experimental treatments and testing significant differences between mean characteristics attained by Duncan´s multiple rang test (Duncan 1955).

**RESULTS AND DISCUSSION**

**Criteria for growth, weight gain, growth rate, relative and qualitative growth:**

The results of the statistical analysis showed that there were significant differences ($P < 0.05$) and the final average weight for $T_7$ (the mixture 5%) was 52.95 g compared to the rest of the treatments. It was noted that there were no significant differences between $T_2$, $T_3$ and $T_4$ respectively. $T_6$ (mixture 2.5%) exhibited the lowest average weight and reached 42.77 g. The results of the statistical analysis also showed that there was a significant difference ($p < 0.05$) in the rate of total and daily weight gain. $T_2$, $T_3$, $T_4$ and $T_7$ outperformed $T_1$ (control) and the best was for $T_7$ since the rate of increase was 5% with a total weight of 34.19 g fish$^{-1}$ and a daily weight increase of 1.08 g fish$^{-1}$ on average. The results of the relative growth showed that there were no significant differences between $T_2$, $T_3$ and $T_4$ respectively, while $T_7$ was significantly different ($P \leq 0.05$) amounting to 182.23% and was higher than $T_1$ (control) and $T_6$. The results of the specific growth rate showed that there were significant differences ($P \leq 0.05$) between $T_7$ compared to the control and the rest of the treatments, which amounted to 2.11%. There were also no significant differences between $T_2$, $T_3$ and $T_4$ respectively (Table 3). Al-Araji (2020) reported higher values of final weight as well as total and daily increase in feeding common carp once using Azolla plant with substitution ratios of 2.5 and 5%, respectively, as a substitute for soybeans, which is consistent with the current study's findings. Mohapatra *et al.* (2013) indicated that the best growth rate and weight gain for fingerlings of common carp (*Cyprinus carpio*) when replacing duckweed plant (*Lemna minor*) instead of fishmeal at percentages of 0% and 15%, but a decrease in the rate of growth and weight gain when using duckweed by 45%. this is in agreement with Asimi *et al.* (2018) who replaced duckweed by 15% and reched better results for growth and weight gain compared to higher inclusion levels (30% and 45%). It was observed in our current study that increasing the proportion of azolla substitution for more than 5% in the experimental diets of common carp may lead to a decrease in the rate of weight gain and growth rate similar to results obtained by Sahu (2006). The decrease in growth due to the increase in the concentration of Azolla in the diets is due to the imbalance of amino acid composition in Azolla protein because it is high in methionine and lysine compared to soybean meal, meat and bone meal (Das *et al.* 2018). The results of our research in Table 3 agreed with Herawati *et al.* (2020) in feeding tilapia fish on duckweed at a rate of 2.5% instead of soybeans, which exhibited the best growth performance for fish and gave the highest total weight gain of 33.03 g fish$^{-1}$. The highest relative growth rate was 2.01% because the plant contains a high percentage of the amino acids such as lysine and arginine acid in higher proportions than soybeans. This also is in agreement with results of Aslam *et al.* (2017) who reported significant rise in the final weight gain of fingerlings of grass carp fish. In a diet containing water lentils, it amounted to 15.84 g, compared to the feeding diet of soybeans (12.63 g). The results of the statistical analysis showed an increase in the values of growth criteria for fish in $T_7$ compared to the rest of treatments because Azolla and duckweed contain high levels of protein and essential amino acids such as lysine and methionine, while the low rate (%) of fibers that makes them more useful in feeding carp. It provides it with all its needs of essential amino acids, and this is inline with results of Hugel (2020) on azolla and water lentils in feeding fish.

**Food intake, feed conversion ratio, and feed efficiency ratio**

The criteria for benefiting from food included the food intake, the food conversion factor and the food efficiency ratio, and a significant increase ($p < 0.05$) was noted in the food intake rate and the food efficiency ratio $T_7$ (5%

mixture), which reached 51.13 g fish$^{-1}$ and 70.86%, respectively, compared to $T_1$ (control; 47.23 g fish$^{-1}$ and 59.35%), and also $T_2$ (Azolla 2.5%; amounting to 43.63 g fish$^{-1}$ and 46.31% respectively). Also, significant difference ($p < 0.05$) was found between $T_3$ (Azola 5%; amounting to 2.19) and all other treatments (Table 4).

**Table 3.** Effect of substituting different levels of azolla, duckweed and their mixture on common carp growth parameters, weight gain, growth rate, percentage growth, and specific growth.

| **Studied criteria** / **Treatments** | **Initial weight (g fish$^{-1}$)** | **Final weight (g fish$^{-1}$)** | **Total weight gain (g fish$^{-1}$)** | **Daily increase (g fish$^{-1}$)** | **Percentage growth %** | **Specific growth rate** |
|---|---|---|---|---|---|---|
| Control (1) | a 18.68 ± 0.02 | b 46.74 ± 1.92 | c 28.06 ± 1.91 | c 0.95 ± 0.04 | c 150.2 ± 10.1 | c 1.87 ± 0.08 |
| Azolla (2.5%) (2) | a 18.67 ± 0.1 | ab 50.1 ± 0.61 | ab 31.42 ± 0.51 | ab 1.02 ± 0.01 | ab 168.07 ± 1.81 | ab 2.01 ± 0.01 |
| Azolla (5%) (3) | a 18.86 ± 0.12 | ab 51.34 ± 0.72 | ab 32.74 ± 0.60 | ab 1.04 ± 0.01 | ab 172.12 ± 2.08 | ab 2.04 ± 0.01 |
| Duckweed (2.5%) (4) | a 18.69 ± 0.1 | ab 51.2 ± 2.10 | ab 32.47 ± 2.21 | ab 1.08 ± 0.04 | ab 174.12 ± 12.75 | ab 2.05 ± 0.09 |
| Duckweed (5%) (5) | a 18.85 ± 0.05 | bc 47.53 ± 0.65 | bc 28.66 ± 0.70 | bc 0.95 ± 0.01 | bc 151.89 ± 4.10 | bc 1.88 ± 0.03 |
| Mixture (2.5%) (6) | a 18.64 ± 0.08 | c 42.77 ± 0.15 | c 24.08 ± 0.06 | c 0.87 ± 0.004 | c 128.93 ± 0.35 | c 1.69 ± 0.02 |
| Mixture (5%) (7) | a 18.76 ± 0.16 | a 52.95 ± 0.60 | a 34.19 ± 0.44 | a 1.08 ± 0.01 | a 182.23 ± 0.79 | a 2.11 ± 0.06 |

*The various letters within the single column of the studied trait indicate significant differences at probability level ($P \leq 0.05$).

**Table 4.** Effect of substituting different levels of Azolla, duckweed and mixture (Azolla and duckweed) on food intake parameters, feed conversion factor and food efficiency ratio for common carp (mean ± standard error).

| **Studied criteria** / **Treatments** | **Food intake (g fish$^{-1}$)** | **Feed conversion ratio** | **Feed efficiency ratio %** |
|---|---|---|---|
| Control (1) | b 47.23 ± 0.86 | b 1.68 ± 0.08 | b 59.35 ± 2.96 |
| (2.5%) Azolla (2) | c 43.63 ± 2.14 | b 1.64 ± 0.11 | c 46.31 ± 6.03 |
| (5%) Azolla (3) | ab 48.17 ± 0.68 | a 2.19 ± 0.28 | ab 61.32 ± 4.20 |
| Duckweed (2.5%) (4) | ab 48.77 ± 0.51 | b 1.58 ± 0.10 | ab 63.61 ± 4.10 |
| Duckweed (5%) (5) | b 46.71 ± 0.25 | b 1.62 ± 0.002 | ab 61.54 ± 0.06 |
| Mixture (2.5%) (6) | ab 48.08 ± 0.77 | b 1.44 ± 0.45 | ab 69.26 ± 2.05 |
| Mixture (5%) (7) | a 51.13 ± 0.30 | b 1.41 ± 0.05 | a 70.86 ± 2.82 |

The various letters within the single column of the studied trait indicate significant differences at probability level ($P \leq 0.05$).

The results of the statistical analysis showed an increase in the values of the feed ration in $T_7$ in the quantity of feed supplied and the food efficiency ratio compared to the rest of treatments, because Azolla and duckweed contain high levels of protein and essential amino acids such as lysine and methionine, and the lack of fiber makes

them more useful in feeding carp. This is in agreement with Hugel (2020) who worked on Azolla and water lentils in feeding fish. Asimi *et al.* (2018) reported a decrease in the feed conversion factor of duckweed compared with the control diet in the feeding of common carp, when different percentages of water lentils were replaced in the diets as a partial substitute for fish meal. The ratios of the feed conversion factor of water lentil and control diet were 2.95 and 2.45 respectively. The high food conversion factor due to the upraised concentration of Azolla compared to the diets free of Azolla could be explained by the presence of dietary fibers and ash. The highest percentage of the food conversion factor of Nile tilapia (4.2) fed on diets enriched with dried Azolla (*A. pinnata*) was at a level of 25% (Abdel-Tawwab 2008). When high levels of Azolla are included, the digestibility and consumption of feed are deteriorated by affecting the activity of digestive enzymes, and thus growth performance correlates with improved nutrient digestibility and feeding efficiency (Hong *et al.* 2004; Sithara & Kamalaveni 2008).

**Protein intake, protein efficiency ratio, precipitated protein, and protein productive value:**

The results of the statistical analysis indicated that there were significant differences ($p < 0.05$) between the $T_7$, which was significantly higher compared to $T_1$ (control), and their values were 13.09 and 11.95 (g $fish^{-1}$) , respectively (Table 5). There were no significant differences for the ingested protein between $T_2$, $T_3$ and $T_4$ , $T_5$ and $T_6$, respectively, while the results of the statistical analysis in the percentage of protein efficiency showed a significant difference in $T_7$ (amounted to 2.77) compared to $T_1$ (control), which exhibited less value (2.31). The precipitated protein gave a significant superiority ($p < 0.05$) in $T_2$ (Azolla 2.5%) and $T_6$ (mixture 2.5%), which amounted to 7.25 and 6.58 g $fish^{-1}$ respectively, compared to $T_1$ (control). There were also no significant differences between $T_3$, $T_4$, $T_5$ and $T_7$), respectively. In the case of the protein-producing value, significant differences ($p < 0.05$) were found in $T_2$ (58.70%), compared to the rest of treatments (Table 5). Al-Araji (2020) reported a significant increase in the value of the precipitated protein and the protein-producing value of carp fed on Azolla 2.5%, where their value reached 1.42 g $fish^{-1}$ and 25.78%, respectively, in agreement with the results of our study. The current results showed the high values of protein intake and the protein efficiency ratio in $T_7$ compared to the rest of the experimental diets, since Azolla and duckweed contain high levels of protein and essential amino acids such as lysine and methionine and a low percentage of fiber, which makes them more useful in feeding carp. These items give fish all needs in essential amino acids, and this is what Hugel (2020) agreed upon in his study on Azolla and duckweed in feeding carp.

**Table 5.** Effect of substitution of different levels of Azolla, duckweed and their mixture (Azolla and duckweed) on the parameters of the protein intake, protein efficiency ratio, precipitated protein, and protein productive value of common carp (mean ± standard error).

| **Studied criteria** / **Treatments** | **Protein intake (g $fish^{-1}$)** | **Protein efficiency ratio** | **Precipitated protein (g $fish^{-1}$)** | **protein productive value (%)** |
|---|---|---|---|---|
| Control | b | b | bc | a |
| (1) | 11.95 ± 0.22 | 2.31 ± 0.11 | 4.93 ± 0.11 | 40.80 ± 0.21 |
| (2.5%) Azolla | ab | ab | a | a |
| (2) | 12.36 ± 0.03 | 2.56 ± 0.04 | 7.25 ± 0.44 | 58.70 ± 3.75 |
| (5%) Azolla | ab | ab | ab | a |
| (3) | 12.33 ± 0.17 | 2.39 ± 0.16 | 5.80 ± 0.21 | 47.03 ± 1.06 |
| Duckweed (2.5%) | ab | ab | ab | b |
| (4) | 12.48 ± 0.13 | 2.48 ± 0.16 | 5.93 ± 0.69 | 47.44 ± 5.01 |
| Duckweed (5%) | ab | ab | ab | b |
| (5) | 12.59 ± 0.21 | 2.64 ± 0.11 | 6.25 ± 0.72 | 49.52 ± 4.48 |
| Mixture (2.5%) | ab | ab | a | ab |
| (6) | 12.31 ± 0.20 | 2.71 ± 0.08 | 6.58 ± 0.29 | 53.44 ± 1.48 |
| Mixture (5%) | a | a | ab | b c d |
| (7) | 13.09 ± 0.08 | 2.77 ± 0.11 | 6.04 ± 0.52 | 46.12 ± 3.72 |

The various letters within the single column of the studied trait indicate significant differences at probability level ($P \leq 0.05$).

Velásquez-Cruz *et al.* (2014) observed a significant increase in the protein efficiency ratio when feeding piranha (*Piaractus brachypomus*) on rations containing Azolla (*Azolla filiculoides*) and Duckweed (*Spirodela polyrhiza*) by 15% and their value was 2.78 and 2.66, respectively, compared to the control diet (2.35). This is due to the high levels of protein and essential amino acids in Azolla and duckweed. Increasing the concentration of Azolla above 10% with an accompanying decrease in soybean meal lead to a deficiency of essential amino acids and thus

a decrease in protein quality. Santiago *et al.* (1988) and Olvera-Novoa *et al.* (1990) reported that this phenomenon is similar to using alfalfa and *Azolla pinnata* in tilapia diets. This is in agreement with the results of Fasakin & Balogun (1998) about the decreased efficiency of protein with an upraised amount of Azolla in the feeding ration of *Clarias gariepinus*.

**The chemical composition of the edible part of the fish**

The results of the statistical analysis of the chemical composition of the fish muscle in the percentage of protein showed that there were significant differences ($p < 0.05$) between the treatments, where the percentage of protein in $T_7$ increased significantly over the rest of the treatments and amounted to 19.87%. No significant differences were found in protein ratios in T2, T3 and T6 respectively. There were also no significant differences in fat and ash ratios among all treatments except for $T_1$ in ash ratio, which recorded a significant decrease ($p < 0.05$) compared to the rest of the treatments (amounted to 1.89%). Fuethermore, the moisture percentage recorded a significant decrease ($p < 0.05$) in $T_7$ compared to the rest of the other treatments (amounted to 69.22%; as shown in Table 6. Our results in Table 6 indicate an increase in the protein content in the body of carp in $T_7$, as a result of the Azolla and water lentils containing a good percentage of protein, as well as an increase in the amino acids levels of lysine and methionine, which makes them easy to accept and palatable by the fish. This is in agreement with Hugel (2020) about Azolla and duckweed in feeding fish. Antoine *et al.* (1986) noted that when feeding *O. Niloticus* and *C. melanorum* on Azolla, they had high moisture content and low body fat concentrations. El-Sayed (1992) explained that body protein and fat were negatively associated with high levels of Azolla in fish feed rations, while the association was positive for ash and body moisture, and this contradicts the data of crude body protein in our current study. El-Sayed (1992) agrees with regard to fat and ash content and body moisture, and between Micha *et al.* (1988) that adding Azolla at increased levels to fish diets of *O. niloticus* and *T. rendalli* led to a decrease in body fat percentage for both species and no effect in crude protein content. Das (2018) reported that once adding different levels of Azolla to the diet of *Barbonymus gonionotus* fish from Thai silver, the crude fat, protein and ash displayed a negative relationship with the elevation in the concentration of Azolla in the diet and moisture. Yalmiz *et al.* (2004) noticed a significant increase ($p < 0.05$) in the muscle protein (%) of common carp when fed with 20% duckweed compared to other treatments. The protein percentage was 16.60%, while the fat content was high for fish fed with 10 and 15%, duckweed respectively. A significant decrease in ash (%) in treatments fed on duckweed was recorded by 10% (Yalmiz *et al.* 2004).

**Table 6.** Effects of substitution of different proportions of Azolla and duckweed and their mixture (%) on the chemical composition of the edible portion of common carp (mean ± standard error).

| **Treatments** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Studied criteria** | **Before the experiment** | **Control (1)** | **Azolla (2.5%) (2)** | **Azolla (5%) (3)** | **Duckweed (2.5%) (4)** | **Duckweed (5%) (5)** | **Mixture (2.5%) (6)** | **Mixture (5%) (7)** |
| Crude protein % | 16.33 ± 0.13 | b 17.10 ± 0.40 | ab 17.86 ± 1.05 | ab 18.84 ± 0.90 | b 16.41 ± 0.29 | b 16.50 ± 0.85 | ab 18.00 ± 0.88 | a 19.87 ± 0.71 |
| Raw fat % | 6.91 ± 0.06 | a 7.79 ± 1.41 | a 7.22 ± 0.72 | a 7.14 ± 0.97 | a 7.85 ± 0.31 | a 7.17 ± 0.19 | a 8.24 ± 0.70 | a 7.68 ± 0.43 |
| Ash % | 3.53 ± 0.03 | b 1.89 ± 0.002 | a 3.65 ± 0.08 | a 3.53 ± 0.36 | a 3.61 ± 0.13 | a 3.36 ± 0.12 | a 3.71 ± 0.01 | a 3.23 ± 0.12 |
| Humidity % | 73.94 ± 0.04 | ab 73.22 ± 1.00 | b c d 71.26 ± 0.41 | cd 70.45 ± 0.29 | a b c 72.11 ± 0.74 | ab 72.69 ± 1.18 | cd 70.04 ± 0.18 | c 69.22 ± 0.19 |
| Dry matter % | 26.06 ± 0.04 | bc 26.78 ± 0.04 | a b c 28.73 ± 0.41 | ab 29.54 ± 0.29 | b c d 27.88 ± 0.74 | bc 27.31 ± 1.17 | ab 29.95 ± 0.18 | a 30.78 ± 0.19 |

*The different letters within the same row of the studied trait indicate significant differences at the probability level ($P \leq 0.05$).

## CONCLUSION

It can be concluded from this study that the addition of the mixture (Azolla and duckweed) by 5% in the diet of carp exhibited a positive effect on the growth parameters. It was observed that the fish benefited from a mixture of 5% Azolla and duckweed by improving the lumen of the digestive system and thus enhancing the values of feed intake, the food efficiency ratio and the food conversion factor., The mixture (5%) exhibited an increase in the protein intake and the efficiency of the protein, because the Azolla and duckweed are considered as a good and inexpensive sources of protein compared to other protein sources that are expensive because of import and are not produced locally. The productivities of the Azolla plant and duckweed are characterized by their high production of biomass and also tolerance to different environmental conditions.

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