Effect of The Type of Solvent in Azolla Extracts Added to The Ratio on The Growth of Common Carp Cyprinus Carpio L.

Ahmed Khalaf Abd and Nidal Tahseen Taha

1,2College of Agriculture and Forestry, University of Mosul, Iraq.

Email: ahmed.agp24@student.uomosul.edu.iq

Abstract

The study was conducted in the fisheries laboratory of the Department of Animal Production in the College of Agriculture and Forestry / the University of Mosul. The experiment included feeding common carp fish using ten experimental diets containing azalea plant extract in proportions (0.5, 1, 1.5%) of each solvent, ether, acetone, and ethanol, and the control was free of additives. Glass tanks were used in the growth experiment of carp fish for a period of 49 days. The results of the statistical analysis of the final weight values, total and daily weight gain (gm/fish), and the relative and qualitative growth rate % showed that there were significant differences (P≤ 0.05) for the experimental treatments, and the third treatment (ether 1%) was significant compared to the rest of the treatments. While the results of the analysis of the amount of food consumed and the ratio of food efficiency in the presence.

Keywords: Azolla extract, Active compounds, Anti-nutritional.

1.Introduction

Attention is directed in our time towards fish farming due to the increasing demand for consumption of fish meat because of its great advantages compared to the rest of animals, as its meat is considered integrated healthy food and the protein in its meat is of good quality because it contains essential amino acids, unsaturated fatty acids and vitamins, and mineral elements and salts [1]. The culture of common carp Cyprinus carpio L. has received wide attention due to its high production rates, rapid growth, clear resistance to adverse environmental conditions, ease of cultivation, and availability of its requirements, which are typical of fish to be cultured on a commercial scale [2]. The development in fish farming has led to an increase in fish production inputs, in particular the feeding process, which constitutes more than 50% of the cost of fish farming. Therefore, it is necessary to search for different alternatives to replace the costly feed ingredients such as fish meals and soybean meals [3]. These alternatives are Azolla sp plant extract because of its active compounds that contribute to increasing growth and raising healthy immunity in fish.

Among these alternatives was Azolla extract, which contains male bioactive substances [4] with a ratio of phenols (90.2 mg/g), tannins (82.2 mg/g), flavonoids (52.5 mg/g), and saponins (12.1 mg/g), g) and alkaloids (2.2 mg/g) in Azolla. Azolla plant contains protein 19-30% of the dry weight (Peters, 1977) and it is a protein source that contains most of the essential amino acids and minerals iron, calcium, magnesium, potassium, phosphorous, and manganese [5]. highly nutritious [6,7]. The Azolla plant is characterized by its ease of cultivation, high yield, good nutritional value, and effective effect in preventing the oxidation of meat fats [8]. The study aimed to add the fatty extract of Azolla plant with three non-polar, partial and polar organic solvents in different proportions to the diets of experimental carp fish and to show the extent to which carp fish benefited from growth parameters, food utilization efficiency, and the chemical composition of fish bodies from the active substances present in the Azolla plant.

2. Materials and Methods

The study was conducted in the fish laboratory of the Department of Animal Production at the College of Agriculture and Forestry / the University of Mosul for a period of seven weeks, from 3/12/2020 to 21/1/2020 using 20 glass tanks with dimensions (40 x 60 x 40) cm placed On steel bearings of three floors, each tank was equipped with an air pump type (RS-510) of Chinese origin, with feeding all tanks air supply tubes from an AUTO SAN type air compressor of Chinese origin.
Fingerlings of common carp fish, Cyprinus Carpio L., were used in the growth experiment, which was placed in ponds containing saline solution at a concentration of 3 g/L for 5 minutes until signs of stress appeared on the fish to get rid of bacteria and external parasites, if any [9]. 140 fingerlings of carp fish with an average weight of ±18.59 g/fish were distributed to 20 glass tanks with seven fish/ponds and two replicates/treatments. These basins were equipped with liquefied water by means of a large tank inside the laboratory in which the water was stored for 24 hours to ensure that it is free of chlorine and to obtain water at a moderate temperature. The laboratory temperature was controlled between (25-30) °C using air conditioners.

Waste, waste, and food residues not eaten by the fish were disposed of in the ponds on a daily basis by partially replacing the pond water by siphoning 20-25% of the pond water and adding pure water from the water tank in the laboratory, and the fish were fed twice a day during the acclimation period until Start the search experience.

The water temperature of the glass tanks was measured with a mercury thermometer, and it was at a rate of 24 °C, to ensure a suitable temperature for the growth of common carp fish, which is 25 °C, because it is a warm water fish [7]. As for the degree of pH, it was between 7.7 - 8.1 and it was measured by a pH meter type LABTECH (DIGITAL pH METER). It is within the recommended limits [10].

The primary feed materials were brought to the fish laboratory and ground by a laboratory mill of German origin, and ten experimental rations were made by adding the fat extract of the Azolla plant to the experimental rations in different proportions (0%), comparison and (0.5%, 1%, 1.5%) fat extract mediated by a non-polar solvent. Ether and (0.5%, 1%, 1.5%) lipid extract mediated by partial polar solvent acetone and (0.5%, 1%, 1.5%) lipid extract mediated by polar ethanol solvent for treatments (1, 2, 3, 4, 5, 6, 7, 8, 9, 10) respectively, as shown in Table (1). The proportions of the feed materials were mixed well for the purpose of homogeneity of the mixture for each ration separately. A cup of warm water was added to the mixture, then the mixture was placed in a National meat mincer machine (Japanese origin with holes 4 mm), where small and cohesive strips were formed and dried in the laboratory for three days It was cut into small pieces to fit the size of the mouth of the experimental fish and placed in opaque bags to prevent exposure to light and kept inside plastic containers.

The relationships are classified as follows:

- Control diet (1): Free from Azolla extract.
- Diet (2): Adding a fatty extract of the Azolla plant extracted by a nonpolar petroleum ether solvent at a rate of 0.5% to the total diet.
- Diet (3): Adding a fatty extract of the Azolla plant extracted by means of a nonpolar petroleum ether solvent at a rate of 1% to the total diet.
- Diet (4): Adding a fatty extract of the Azolla plant extracted by a nonpolar petroleum ether solvent at a rate of 1.5% to the total diet.
- Diet (5): Adding a fatty extract of the Azolla plant extracted by partial polar solvent acetone at a rate of 0.5% to the total diet.
- Diet (6): Adding a fatty extract of Azolla plant extracted with partial polar solvent acetone at a rate of 1% to the total diet.
- Diet (7): Adding a fatty extract of Azolla plant extracted with partial polar solvent acetone at a rate of 1.5% to the total diet.
- Diet (8): Adding a fatty extract of the Azolla plant extracted by polar solvent ethanol at a rate of 0.5% to the total diet.
- Diet (9): Adding a fatty extract of the Azolla plant extracted by 1% ethanol polar solvent to the total diet.
- Diet (10): Adding a fatty extract of the Azolla plant extracted by means of polar solvent ethanol at a rate of 1.5% to the total diet.

The experimental fish were fed the experimental diets mentioned in Table (1) at a rate of 3-5% of their body weight, and the system of providing three meals a day from the ration was adopted. The amount of ration provided to the experimental fish was increased depending on the weight gain that occurred from the growth of the fish, taking the weights of the fish every two weeks Using a sensitive electronic scale (0.01) gm, a type of Citizen of Chinese origin, for a period of seven weeks, the feed provided to the fish was cut off one day per week to increase the fish's appetite for feeding for the next day during the experiment period.
Table 1. Composition of the components of the experimental diets (%) resulting from adding different percentages of the fatty extract of the azalea plant with different solvents.

| Transactions feed ingredients | Control | Ether 0.5% | Ether 1% | Ether 1.5% | Acetone %0.5 | Acetone %1 | Acetone %1.5 | Ethanol %0.5 | Ethanol %1 | Ethanol %1.5 |
|------------------------------|---------|------------|---------|-----------|-------------|-----------|-------------|-------------|-----------|-------------|
| Fish meal                    | 12      | 12         | 12      | 12        | 12          | 12        | 12          | 12          | 12        | 12          |
| soybean meal                 | 30      | 30         | 30      | 30        | 30          | 30        | 30          | 30          | 30        | 30          |
| wheat bran                   | 19      | 19         | 19      | 19        | 19          | 19        | 19          | 19          | 19        | 19          |
| yellow corn                  | 16.5    | 16.5       | 16.5    | 16.5      | 16.5        | 16.5      | 16.5        | 16.5        | 16.5      | 16.5        |
| Local black barley (binding substance) | 20 | 20         | 20      | 20        | 20          | 20        | 20          | 20          | 20        | 20          |
| A mixture of vitamins, minerals, and salts | 0.5 | 0.5        | 0.5     | 0.5       | 0.5         | 0.5       | 0.5         | 0.5         | 0.5       | 0.5         |
| Salt                         | 1       | 1          | 1       | 1         | 1           | 1         | 1           | 1           | 1         | 1           |
| Limestone                    | 0.5     | 0.5        | 0.5     | 0.5       | 0.5         | 0.5       | 0.5         | 0.5         | 0.5       | 0.5         |
| Petroleum Ether              | -       | 0.5       | 0.5     | 1         | 0.5         | 1         | 1           | 0.5         | 1         | 1           |
| Acetone                      | -       | -         | -       | 0.5       | 1           | 1         | 1           | -           | -         | -           |
| Ethanol                      | -       | -         | -       | -         | -           | -         | -           | 0.5         | 1         | 1.5         |

Table 2. Chemical composition (%) of experimental diets based on the dry weight.

| chemical composition | Transactions | First | second | third | Fourth | Fifth | Sixth | Seven | eight | ninth | Tenth |
|----------------------|--------------|-------|--------|-------|--------|-------|-------|-------|-------|-------|-------|
| raw protein          | 25.60        | 25.60 | 25.60  | 25.60 | 25.60  | 25.60 | 25.60 | 25.60 | 25.60 | 25.60 | 25.60 |
| raw fiber            | 4.70         | 4.70  | 4.70   | 4.70  | 4.70   | 4.70  | 4.70  | 4.70  | 4.70  | 4.70  | 4.70  |
| ether extract        | 4.10         | 8.95  | 10     | 8.91  | 7      | 9.45  | 10.5  | 10.5  | 10.5  | 10.5  | 10.5  |
| Ash                  | 6.97         | 6.97  | 6.97   | 6.97  | 6.97   | 6.97  | 6.97  | 6.97  | 6.97  | 6.97  | 6.97  |
| Humidity             | 7.20         | 7.20  | 7.20   | 7.20  | 7.20   | 7.20  | 7.20  | 7.20  | 7.20  | 7.20  | 7.20  |
| Nitrogen-free extract (NFE) | (Mica Joule/Kilogram) (Metabolic energy) | 13.28 | 14.24 | 14.45 | 41.09  | 13.85 | 14.34 | 14.54 | 14.54 | 14.55 | 14.55 |

* Metabolic energy was calculated based on [39], which is: 13.8 x NFE + 33.5 x Fat + 18.8 x Protein = (Kg/MJ)ME

3. Methods of Measuring The Growth Parameters of Experimental Fish

The criteria for measuring the growth of fish were adopted to show the effect of substituting Azolla as a partial substitute for soybean meal on their growth, represented by calculating the total weight gain of fish (TWG) [11], and the growth rate of fish (Growth Rate (GR) [12], Fish Relative Growth Rate (RGR) [13], Specific Growth Rate (SGR) for Fish [14] and Fish Feed Conversion Ratio (FCR) [15], the Feed Efficiency Ratio (FER) for fish [16], the Protein Efficiency Ratio (PER) for fish [17], the protein intake for fish, and the PPV Protein Productive Value for fish. [18], according to the following equations:

Total weight gain (g/fish) = Final weight (g) – Initial weight (g)

Daily weight gain (g/fish) = \[
\frac{\text{Final weight} - \text{Initial weight}}{\text{Number of days}}
\]

Daily growth rate (g/fish/day) = \[
\frac{\text{Weight gain (g)}}{\text{Duration of experiment (days)}}
\]

Specific growth rate = \[
\frac{\log_{10} \text{final weight (g)} - \log_{10} \text{initial weight (g)}}{\text{Duration of experiment (days)}} \times 100
\]

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.

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.
3.1 Chemical analyzes of fish growth experiment (Nutrient components)

The nutrient components (protein, fat, ash, moisture, and nitrogen-free extract) present in the dry matter were estimated:

- Components of processed fish feed.
- The edible part of the body of the experimental research fish.

The nutrients in fish diets and body were estimated on the basis of dry weight based on the approved standard methods (AOAC, 2000) in the estimation of protein, fat, ash, and moisture, and according to the nitrogen-free extract mathematically by the difference method as shown in [43 and Shu, 1989] and as follows:-

- Soluble carbohydrates = 100 – (crude protein % + fat % + ash % + fiber % + moisture %).

The data were analyzed statistically by using the Complete Randomized Design (CRD) by the Statistical Package for Social Science (SPSS 2001) in analyzing the effect of experimental transactions and testing the significant differences between the averages of the studied traits by Duncan’s multiple range test. Test [19].

4. Results and Discussion

Criteria for growth, weight gain, growth rate, relative and qualitative growth: The results of the statistical analysis of the final weight, the total weight gain, the daily increase (gm/fish), and the relative growth in % showed that there were significant differences (P ≤ 0.05) between the experimental treatments shown in Table (15), and the third treatment differed, ether 1%, which amounted to (54.79, 36.24, 1.12), g/fish (195, 44%) for the above traits significantly with the first (control), fifth (acetone 0.5%) and tenth (ethanol 1.5%), while no significant differences were observed between the treatments of ether (0.5%, 1.5%) and acetone. (1%, 1.5%) and ethanol (0.5%, 1%), and there were no significant differences between the control treatments and 0.5% acetone, and the results showed that all treatments were superior to the tenth treatment, ethanol 1.5%, which amounted to (38.90, 20.33, 0.79 g/fish), (109.24%) for the above traits.

The results of the statistical analysis of the specific growth rate showed that there were significant differences (P ≤ 0.05) between the treatments, where the third treatment, ether 1%, and amounted to (2.21), was significantly superior to the rest of the treatments, and it differed significantly with the treatment (control) and the tenth treatment (ethanol 1.5%) and amounted to (1.87). And 1.49), respectively, and it was found that there were no significant differences between the ether treatment (0.5%, 1.5%), the acetone treatment (0.5%, 1%, 1.5%), and the ethanol treatment (0.5%, 1%), and all treatments outperformed The tenth treatment (ethanol 1.5%) amounted to (1.49).

Plant extracts are used in the field of health care for fish breeding and production because they contain effective biological compounds with high nutritional value and are beneficial to the immune system [20] and work to promote growth and antioxidants [21] and is of high benefit to the digestive system and increases appetite [22, 23] and works to protect the liver from diseases and increase its immunity [24, 25].

Table 3. The effect of adding the fatty extract of the Azolla plant using three solvents in different proportions to the feeding rations and its effect on the growth characteristics of common carp fish (mean ± standard error).

| studied standard Transactions | Starting weight (g/fish) | Final weight (g/fish) | Total weight gain (gm/fish) | daily increase (g/fish) | %growth | specific growth rate |
|------------------------------|--------------------------|----------------------|---------------------------|------------------------|---------|---------------------|
| Control (1)                  | 0.01±18.68               | 1.92±46.74          | 1.91±28.06                | 0.04±95                | 10.10±150.20 | 0.08±1.87           |
| Ether 0.5% (2)               | a                        | b                    |                           | b                      | b        | b                   |
| Ether 1% (3)                 | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Ether 1.5% (4)               | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Acetone 0.5% (5)             | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Acetone 1% (6)               | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Acetone 1.5% (7)             | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Ethanol 0.5% (8)             | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Ethanol 1% (9)               | a                        | ab                   | a                         | ab                     | ab       | ab                  |
| Ethanol 1.5% (10)            | a                        | ab                   | a                         | ab                     | ab       | ab                  |

* Different letters in the same column indicate significant differences (P ≤ 0.05).
It was observed from the study that when the concentration of Azolla plant extract increased by (1.5%) in the diet components, the treatments did not outperform the control, due to the presence of some anti-nutritional substances in the extract that affect growth and weight gain. Plant extracts affect the growth of fish and depend on the concentration of the extract. Existing in the composition of the diet, where the growth increases to a certain level of the nutritional extract, and when the concentration increases, it affects the growth of fish.

The best concentration of Azolla plant fat extract that can be used in feeding experimental fish is 1% for each of the solvents ether, acetone, and ethanol. Therefore, the concentration of Azolla plant fat extract should not exceed 1% in feeding fish because it contains a high percentage of anti-nutritional. The best level was found in Aloe Vera extract 2%/kg [26] and for Ulva clathrata freeze-dried seaweed extract 1%/kg [27].

The use of high concentrations of the fatty extract of plants in fish diets leads to poor growth, lack of weight gain or no effect on growth due to the presence of a high concentration of anti-nutritional factors (such as saponins and tannins), which have a toxic effect and cause an allergy to fish and thus affect the Fish farming [28,29], saponins prevents the small intestine from absorbing and transporting nutrients through it [30] and works to reduce the amount of digested protein in the body. [31] through the formation of a protein complex as well as saponins, which is considered an indigestible substance [32].

The results of the study agreed with [33] that the best concentration of the fatty extract of the plant Cynodon dactylon was 1%, but it differed in the solvent sequence, where the researcher found that ethanol is the best, followed by acetone and then petroleum as ether in shrimp feeding, and the results of the study differed. With the results of the researcher [34], the best concentration of fatty extract of Eichhornia crassipes and Sargassum cristaefolium was found to be 1.5% of the ethanol solvent to increase the growth parameters of shrimp fish.

It was found that the fatty extract of Aloe Vera, Euphorbia hirta, Azadirachta indica, Carica papaya and Cinnamomum camphora with ethanol solvent 2 g/kg [35,36] increased weight and increased the rate. Qualitative growth in a tilapia fish because it contains useful active compounds and improves the environment of the digestive system. Reported [37], [38] mentioned that the concentration of 1% fatty extract of ethanol solvent of Phyllanthus amarus enriched Artemia is better than acetone and ether as it led to an increase in weight and growth rate in shrimp fish due to its beneficial active compounds. [39] indicated that the fatty extract of water hyacinth Eichhornia crassipes and marine brown algae Sargassum cristaefolium with a concentration (1.5%) of the ethanol solvent used in shrimp fish diets led to an increase in weight and specific growth rate. It was reported [40] that the extract of Cynodon dactylon with 1% ethanol solvent gave the best results compared to acetone and ether and increased body weight and specific growth rate in shrimp.

[41], indicated in a study of the fatty extract of Alternanthera sessilis, the best concentration (1%) of the ethanol solvent was found, which gave the highest increase in weight and specific growth rate of shrimp fish. In a study of the extract of St. John's plant Hypericum perforatum, Origanum vulgare, and Melissa officinalis conducted by [42] it was found that 0.5% of the fatty extract of plants with ethanol gave the highest weight gain and specific growth rate in tilapia fish. [43] reported that the methanol extract from Melissa officinalis increased weight and specific growth rate in trout. [44] reported that coriander seed extract with methanol at 2% concentration gave the highest weight gain and specific growth rate and improved the feed conversion factor of rainbow trout.

4.1 Food Intake, Food Conversion Factor, and Food Efficiency Ratio

The results of the statistical analysis on the food intake, the food conversion factor, and the food efficiency ratio shown in Table (16) showed that there were significant differences (P≤ 0.05) between the treatments in the amount of feed intake. The control treatment, ether (0.5%, 1.5%), acetone (0.5%, 1%, 1.5%) and ethanol (0.5%, 1%) outperformed the tenth treatment, ethanol (1.5%), which reached (43.63 g/fish), and there were no significant differences between the treatments of ether (0.5%, 1.5%), acetone (1%, 1.5%) and ethanol (0.5%, 1%), but there were arithmetic differences between the treatments where the highest was the ninth treatment (ethanol. 1%) which amounted to (49.20 g/fish) and the lowest treatment of the sixth was acetone (1%) and it amounted to (48.08 gm/fish), and it was found that there were no significant differences between the first treatment, the control (47.23 g/fish) and the fifth treatment acetone 0.5% (46.71) g/fish.

Differences (P≤ 0.05), as the tenth treatment (ethanol 1.5%) was significantly superior to the rest of the treatments and amounted to (2.19), and there were no significant differences between the first treatments, control, and ether (0.5%), 1%, 1.5%, acetone (0.5%, 1%, 1.5%) and ethanol (0.5%, 1%), but there are arithmetic differences between the treatments where the highest treatment of the first control occurred and amounted to (1.68) and the lowest third treatment was ether 1% The feed conversion factor was (1.41).

It was noticed in the estimation of the food efficiency ratio that there were significant differences (P≤ 0.05) between the treatments, where the third treatment outperformed the ether 1% significantly compared to the rest of the treatments and amounted to (70.86%), and it was found that all treatments were superior to the tenth treatment (ethanol 1.5%), amounting to (46.31). %, and there were no significant differences between the treatments of ether (0.5%, 1.5%), acetone (0.5%, 1%, 1.5%), and ethanol (0.5%, 1%), but there are arithmetic differences between the treatments, and the sixth treatment was the best (acetone 1%) and amounted to (69.26%) and the lowest seventh treatment was acetone 1.5% and amounted to (61.32%).
The presence of active compounds in plants is associated with intestinal function, gastrointestinal secretions, and nutrient absorption in the animal GI tract [22,29] and antibacterial formation occurs in the intestinal lumen, thus beneficially improving the alimentary canal environment in order to achieve food utilization [28,30].

The use of water hyacinth plant Eichhornia crassipes and Sargassum cristaefolium extracts with a solvent of 1.5% ethanol solvent gave the best feed conversion factor and high protein efficiency due to its active and active compounds that are useful in feeding shrimp fish. The active compounds in plants act as a health stimulant in aquatic nutrition and in turn, are reflected in the metabolism of food within the digestive system [46]. Some vegetable additives improve the palatability of feeds and this increases feed intake [47]. This is related to many of the biological activities of the compounds contained in the extract, such as the antioxidant and antimicrobial effect [31,41].

The improvement in growth performance, the amount of food consumed and the improvement of the feed conversion factor in fish fed on plant extracts is due to the presence of a wide range of feed components that have an immune effect on the health of the organism, such as polysaccharides, which have prebiotic properties, and can increase the digestibility Nutrients, their absorption and assimilation capacity by improving gastrointestinal morphology [19,14], [29], found in a study of the extract of St. John Hypericum perforatum, Origanum vulgare, and Melissa officinalis that 0.5% of plant extract ethanol improved the feed conversion factor of tilapia fish. The methanol extract from the basil plant Melissa officinalis improved the feed conversion factor in trout [32], and the essential oil extracted from wild oregano increased the amount of feed consumed by carp [11]. [36] reported an increase in feed efficiency and feed conversion ratio when using Turbinaria ornata extract with 1.5% methanol solvent used in shrimp feeding was due to the activity of digestive enzymes, including pepsin, amylase, and lipase [20,8].

4.2 Presented protein, protein efficiency ratio, precipitated protein, and protein-producing value

The results of the statistical analysis in Table (17) on the protein intake indicated that there were significant differences (P≤ 0.05) between the treatments, where the third treatment outperformed the ether (1%) and amounted to (13.09) g/fish over the rest of the treatments, and the first treatment (control) and ether outperformed (0.5%, 1.5%), acetone (0.5%, 1%, 1.5%) and ethanol (0.5%, 1%) on the tenth treatment ethanol (1.5%) amounting to (11.17) g/fish, and there were no significant differences between the ether treatments. (0.5%, 1.5%), acetone (1%, 1.5%) and ethanol (0.5%, 1%), but the presence of

Table 4. Effect of adding the fatty extract of the Azolla plant using three solvents in different proportions to the consumed food, the feed conversion factor, and the food efficiency ratio of common carp (mean ± standard error).

| studied standard Transactions | Amount of food intake (gm/fish) | feed conversion factor | Food Efficiency Ratio |
|-------------------------------|---------------------------------|------------------------|-----------------------|
| Control (1)                   | 0.86±47.23                     | 0.08±1.68              | 2.96±59.35            |
| Ether 0.5% (2)                | 0.13±48.30                     | 0.03±1.52              | 1.23±65.69            |
| Ether 1% (3)                  | 0.30±51.13                     | 0.05±1.41              | 2.82±70.86            |
| Ether 1.5% (4)                | 0.51±48.77                     | 0.10±1.58              | 4.10±63.61            |
| Acetone 0.5% (5)              | 0.25±46.71                     | 0.00±1.62              | 0.06±61.54            |
| Acetone 1% (6)                | 0.77±48.08                     | 0.45±1.44              | 2.05±69.26            |
| Acetone 1.5% (7)              | 0.68±48.17                     | 0.11±1.64              | 4.20±61.32            |
| Ethanol 0.5% (8)              | 0.64±48.38                     | 0.00±1.53              | 0.14±65.38            |
| Ethanol 1% (9)                | 0.86±49.20                     | 0.06±1.47              | 2.96±67.81            |
| Ethanol 1.5% (10)             | 2.14±43.63                     | 0.28±2.19              | 6.03±46.31            |

* Different letters in the same column indicate significant differences (P≤ 0.05).

The result of the statistical analysis in the food conversion factor of Azolla plant extract indicated that there were significant [45] reported that the extract of Alternanthera sessilis with 1% ethanol solvent gave the best feed conversion factor and high protein efficiency due to its active and active compounds that are useful in feeding shrimp fish.
significant differences between ether (0.5%, 1.5%) and acetone (0.5%). The results showed in the protein efficiency ratio that there was a significant difference (P≤ 0.05) between the treatments, where the third treatment, ether 1%, was significantly superior to the rest of the treatments and amounted to (2.77), and it was noted that there were no significant differences between ether (0.5%, 1.5%) and acetone (0.5%, 1%, 1.5%) and ethanol (0.5%, 1%), and all treatments outperformed the tenth treatment, ethanol 1.5%, which amounted to (1.81).

**Table 5.** Effect of adding the fatty extract of the Azolla plant using three solvents with different percentages on the protein intake and deposition, the protein efficiency ratio, and the protein-producing value of common carp (mean ± standard error).

| studied standard Transactions | Intake of protein g/fish | Protein Efficiency Ratio | Precipitated protein g/fish | Protein value% |
|------------------------------|-------------------------|--------------------------|----------------------------|----------------|
| Control                      | 0.22±11.95              | 0.11±2.31                | 0.11±4.93                  | 0.21±40.80     |
| Ether 0.5% (2)               | 0.03±12.36              | 0.04±2.56                | 0.44±7.25                  | 3.75±58.70     |
| Acetone 0.5% (5)             | 0.06±11.95              | 0.00±2.40                | 0.38±4.80                  | 2.93±40.13     |
| Acetone 1% (6)               | 0.20±12.31              | 0.08±2.71                | 0.29±6.58                  | 1.48±53.44     |
| Acetone 1.5% (7)             | 0.17±12.33              | 0.16±2.39                | 0.21±5.80                  | 1.06±47.03     |
| Ethanol 0.5% (8)             | 0.16±12.38              | 0.00±2.55                | 0.06±5.69                  | 1.13±46.01     |
| Ethanol 1% (9)               | 0.21±12.59              | 0.11±42.64               | 0.72±6.25                  | 4.48±49.52     |
| Ethanol 1.5% (10)            | 0.55±11.17              | 0.24±1.81                | 0.68±3.96                  | 4.32±35.25     |

* Different letters in the same column indicate significant differences (P≤ 0.05).

The results of the statistical analysis in the percentage of the precipitated protein indicate that there are significant differences (P≤ 0.05) between the treatments, where the second treatment outperformed ether 0.5% and the sixth acetone 1.5% over the rest of the treatments and amounted to (7.25 and 6.58) g/fish, respectively, and the superiority of the ether treatment was noted (1%, 1.5%), acetone (1.5%) and ethanol (0.5%, 1%) on the first treatment as the control, the fifth acetone 0.5%, and the tenth ethanol 1.5%, as well as the absence of significant differences between the treatment of ether (1%, 1.5%), acetone (1.5%) and ethanol (0.5%, 1%).

It was observed from the protein production value that there were significant differences (P≤ 0.05) between the treatments, where the second treatment outperformed ether 0.5% over the rest of the treatments and amounted to (58.70%), and the sixth treatment acetone 1% and the ninth ethanol 1% outperformed the control and ether treatment (1%, 1.5%), acetone (0.5%, 1.5%) and ethanol (0.5%, 1.5%), and the treatment of ether 1.5% and acetone 1.5% were superior to the treatment of ethanol 1.5%, and it was noted that there were no significant differences between the first treatment and ether (1%, 1.5%) and acetone (0.5%, 1.5%) and ethanol (0.5%, 1.5%).

A study conducted by [36] showed that the addition of Cynodon dactylon and Turbinaria ornata extracts, respectively, with 1% ethanol to the feeding ration contributed to an increase in the efficiency of the protein produced in the fish's body. Shrimp and precipitated protein due to the presence of active compounds in plant extract that play an important role in improving the environment of the digestive system and increasing resistance to parasites and pathogenic bacteria. The addition of plant fatty extracts leads to an increase in the protein deposited in the fish body as a result of the contribution of polyphenols in improving the metabolism of fatty acids and sugar in the blood and improving the health of fish and this reinforced the role of the presence of protein, which was reflected in the increase in the deposited protein [37]. The activity of digestive enzymes such as amylase, trypsin, cytochrome c oxidase, and lactate dehydrogenase leads to an increase in the digestion and absorption of nutrients in fish and saponins, but in certain concentrations, can enhance the permeability of the
intestinal membrane to absorb the digested food components and thus increase production and protein deposition and improve fish health [13].

Herbal fat extracts are considered of high nutritional value because they contain active compounds that stimulate the feeding rate and the proportion of food consumption and improve the biochemical components through the process of transcription that leads to an increase in RNA because of the increase in amino acids that contribute to protein synthesis in the body. Shrimp, fish [48]. It was found that adding the fatty extract of brown algae Turbinaria ornate to a methanol solution of 1.5% to the diet significantly improved the digestive enzymes, biochemical components, protein components, amino acids, and fatty acids in fish shrimp [36].

It shows a vital role for the active compounds found in plants as they are used as antibiotics that reduce the effect of pathogenic microorganisms and this leads to the increase of beneficial microorganisms and their settlement in the alimentary canal where they have a positive effect, which is reflected on the health of the organism and benefit from the nutrients found in the ration and thus leads to increased growth and improved efficiency of utilization of food intake.

4.3 The chemical composition of the fish's body

The results of the statistical analysis of the chemical composition of the fish body in the proportion of protein indicated that there were significant differences (P≤0.05) between the treatments, where the proportion of protein in the second treatment, ether, increased by 0.5% significantly over the rest of the treatments and amounted to (20.44)%, and the proportion of protein in the acetone treatment increased by 1% (18.52, 18.37)%, respectively, on the pre-experiment treatment, the ether 1% treatment, and the acetone treatment 0.5% (16.33, 16.54, 16.55)% respectively, and the protein increased in all treatments over the pre-experiment treatment (16.33)%, and it was noted that no significant differences between the first treatment control, ether 1.5% and ethanol (0.5%, 1%, 1.5%), and no significant differences between the third treatment, ether 1% (16.54)% and the fifth acetone 0.5% (16.55)% as well as the absence Significant differences between 1% and 1.5% acetone treatment (18.52, 18.37)% respectively. There are arithmetic differences between the transactions, where the highest percentage of the second transaction was ether 0.5% and it amounted to (3.95)%, and the lowest percentage for the fifth acetone treatment (18.52, 18.37)% respectively, on the pre-experiment treatment increased by 1% (18.52, 18.37)% respectively, on the pre-experiment treatment, the ether 1% treatment, and the acetone treatment 0.5% (16.33, 16.54, 16.55)% respectively, and the protein increased in all treatments over the pre-experiment treatment (16.33)%

Table 6. Effect of adding the fatty extract of the Azolla plant using three solvents in different proportions on the chemical composition (%) of the portion eaten in common carp (mean ± standard error).

| Transactions | Protein | Fat | Ash | Humidity | Dry matter |
|--------------|---------|-----|-----|----------|------------|
|              | before the experiment | after the experiment | | | |
| Control (1)  | 0.40±17.10 | 1.41±7.79 | 0.00±1.89 | 1.00±73.22 | 1.00±26.78 |
| Ether 0.5% (2) | 0.62±20.44 | 0.81±6.57 | 0.73±3.96 | 0.71±69.03 | 0.71±30.97 |
| Ether 1% (3) | 0.41±16.54 | 0.96±7.01 | 0.03±3.71 | 0.52±72.74 | 0.52±27.26 |
| Ether 1.5% (4) | 0.59±18.03 | 0.11±9.16 | 0.03±3.12 | 0.73±69.67 | 0.73±30.32 |
| Acetone 0.5% (5) | 0.68±16.55 | 1.22±8.66 | 0.04±3.06 | 0.49±71.72 | 0.49±28.28 |
| Acetone 1% (6) | 0.05±18.52 | 0.75±8.53 | 0.05±3.38 | 0.75±69.55 | 0.75±30.44 |
| Acetone 1.5% (7) | 0.43±18.37 | 0.80±5.99 | 0.48±3.91 | 0.12±71.71 | 0.12±28.28 |
| Ethanol 0.5% (8) | 0.29±17.38 | 0.38±7.06 | 0.07±3.57 | 0.16±71.99 | 0.16±28.01 |
| Ethanol 1% (9) | 0.63±17.83 | 2.36±7.35 | 0.30±3.32 | 1.43±71.49 | 1.43±28.51 |
| Ethanol 1.5% (10) | 0.11±17.96 | 0.46±8.04 | 0.15±3.10 | 0.42±70.89 | 0.42±29.10 |

* Different letters in the same column indicate significant differences (P≤ 0.05).
The active compounds in plants stimulate intestinal secretions and this leads to improved digestion, absorption, and assimilation of nutrients, and in turn stimulates the transcription process of nucleic acids and protein synthesis in [36]. It turns out that the 0.5% ether extract achieved an increase in the proportion of protein in the fish body, due to the availability of essential amino acids, especially methionine and lysine, which play an important role in building body tissues, forming enzymes and hormones, and being involved in physiological processes in the body [49]. [45] reported that the protease enzyme is able to reduce the effect of trypsin inhibitors and this leads to improved protein digestion and thus increased efficiency of protein utilization [36]. reported that Turbinaria ornate brown algae extract of 1.5% methanol increased protein, fat, dry matter, ash, and body moisture content of shrimp fish. [38] reported that the fatty extract of Phyllanthus amarus artemia nauplii enriched with 1% ethanol led to an increase in the percentage of fat and protein in the body of shrimp fish. [20] indicated that Cynodon dactylon extract of 1% ethanol solvent caused high protein, fat, dry matter, and ash content and decreased body moisture in shrimps. A study conducted by [26] on the extract of Alternanthera sessilis to 1% ethanol solvent showed an increase in the percentage of protein, fat, ash, dry matter, and low moisture content in the body of shrimp fish.

Conclusions

- Adding the fatty extract of the Azolla plant from the non-polar solvent ether 1% in the ratio of carp had positive effects on the growth parameters represented in the final weight, total and daily weight gain, and the relative and qualitative growth rate.
- It was noticed that the fish benefited from the ration containing the fatty extract of the Azolla plant in improving the environment of the digestive system and thus improving the value of the feed conversion factor and the efficiency of food utilization.
- The active compounds resulting from the fatty extraction process of Azolla plants enhanced the vitality of fish in improving their feed consumption and the general health of the role of these compounds in improving fish immunity.

References

[1] Hassan, Hussein Fadel, and Hashem, Dalia Sadad. (2016). Mesopotamian fish parasites. Fuzuli Press - Kirkuk, first edition, 251 p.
[2] Muhaisen, Farhan Damad. (1983). Fish diseases and parasites. Ministry of Higher Education and Scientific Research, General Directorate of Arabization, p. 227 A.O.A.C. (Association of official analytical chemists). (2000). 17th ed V11 USA.
[3] Balaji, K. Jalaludeen, A. Churchil, RR. Peethambaran, PA. and Sethilkumar, S. (2009). Effect of dietary inclusion of azolla (Azolla pinnata) on production performance of broiler chicken. Indian Journal of Poultry Science, 44: 195-198.
[4] Bhavan, P.S., Manickam, N. & Radhakrishnan, S. (2012) Influence of herbal greens, Murraya koenigii, Coriandrum sativum and Menthe arvensis on growth performance of the freshwater prawn Macrobrachium rosenbergii post larvae. Res. J. Biotechnol., 7, p:149–157.
[5] Bilen S, Tarek Abdalsalam Salem Altief, Keriman Yürüten Özdemir, Mohamed Omar Abdalla Salem, Ertugrul Terzi & Kerim Güney. (2020). Effect of lemon balm (Melissa officinalis) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (Oncorhyncus mykiss). Fish Physiology and Biochemistry volume 46, p:471–481.
[6] Citarasu, T. (2010) Herbal biomedicine - a new opportunity for the aquaculture industry Aquac Int., 18, p:403-414.
[7] Crockford, T. and Johnston, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of the common carp (Cyprinus carpio L.). Journal of comparative physiology B, 160(1), 23–30.
[8] Debnath M, Paul AK, Bisen PS (2007) Natural bioactive compounds and biotechnological potential of marine bacteria. Curr Pharm Biotechnol 8, p:253–260.
[9] Dhama K, Chakraborty S, Tiwari R (2013) Panchgavya therapy (Cowpathy) in safeguarding health of animals and humans—a review. Res Opin Anim Vet Sci 3, p:170–178.
[10] Duncan, C.B. (1955). Multiple range and Multiple “ F ” test. Biometric, 11: 1-12.
[11] Farsani, M. N., HoseiniFar, S. H., Rashidian, G., Farsani, H. G., Ashouri, G., & Van Doan, H. (2019). Dietary effects of Coriandrum sativum extract on growth performance, physiological and innate immune responses and resistance of rainbow trout (Oncorhyncus mykiss) against Yersinia ruckeri. Fish & Shellfish Immunology, Volume 91, Pages 233-240.
[12] Food and Agriculture Organization (1981)+(FAO). Report of the symposium on new developments in the utilization of the heated effluents in the circulation system for intensive aquaculture stawner, 29-30. Rome. Italy.
[13] Francis, G., Kerem, Z., Makkar, H.P.S., and Becker, K. 2002. The biological action of saponins in animal systems: A review. Brit. J. Nutr., 88:587–605.
[14] Gabriel, N. N., Qi, J., He, J., Ma, X. Y., Kpundeh, M. D., & Xu, P. (2015a). Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT). Fish & Shellfish Immunology, 44, 504–514.
[15] Gamboa-Delgado, J. Molina-Poveda, C. Cahu C. (2003). Digestive enzyme activity and food ingesta in juvenile shrimp Litopenaeus vannamei (Boone 1931) as a function of body weight. Aquaculture Research 34: 1403–1411.

[16] Gerking, S. D. (1971). Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. Physiological Zoology, 44(1), 9-19.

[17] Gupta M.V.; Dey, M.M. and Penman, D. (2005). Importance of Carp genetic resources. In: Penman, D.J.; Gupta, M.V. and Dey, M.M. (Eds.). Carp genetic resources for aquaculture in Asia. World Fish Center Technical Report 65, Penang, Malaysia: World fish center, 1-5 p.

[18] Gurkan, M., Yılmaz, S., Kaya, H., Ergun, S., & Alkan, S. (2015). Influence of three spice powders on the survival and histopathology of Ochrobactrum mossambicium before and after Streptococcus iniae infection. The Marine Science and Technology Bulletin, 4(1), 1–5.

[19] Dreebe, H.A. (2017). The Impact of Cultivated Area and Price on Production of Rice in AL-Qadisisiyah –Iraq During the Period (1990-2014) by Using VECM. Al-Qadisiyyah Journal for agriculture science, Vol.7, No.1:123-135.

[20] Hidalgo M C, Urea E, Sanz A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquacult., 170: 267-283.

[21] Indira, D. Sarjan Rao, K. Suresh, J. Venugopal Naidu, K. and Ravi, A. (2009). Azolla (Azolla pinnata) as feed supplement in buffalo calves on growth performance. Indian Journal of Animal Nutrition, 26: 345-348.

[22] Irkin, L. C., Yigit, M., Yilmaz, S., & Maita, M. (2014). Toxicological evaluation of dietary garlic (Allium sativum) powder in European sea bass Dicentrarchus labrax juveniles. Food and Nutrition Sciences, 5(11), 989.

[23] Jamroz, D. Jamroz, A. Wiliczkiewicz, T. Wertelecki, J. Orda, J. (2005). Skorupinska Use of active substances of plant origin in chicken diets based on maize and locally grown cereals Br. Poult. Sci., 46, pp. 485-493.

[24] Jobling, M. and Koskela, R. (1996). Inter-individual variation in feeding and growth in rainbow trout Oncorhynchus mykiss during restricted feeding and in a subsequent period of compensatory growth. J. Fish. Biol., 49: 658 - 667.

[25] Johnson FT, Gee JM, Price K, Curi C, Fenwick GR (1986). Influence ofsaponin on gut permeability and active nutrient transport in-vitro.116:2270-2277.

[26] Kalaiselvi, D., Mohankumar, A., Shanmugam, G., Thiruppathi, G., Nivitha, S., and Sundararaj, P. (2018). Altitude-related changes in the phytochemical profile of essential oils extracted from Artemisia nilagirica and their nematicidal activity against Meloidogyne incognita. Ind. Crops Prod. 139:1147-2.

[27] Kanagarasu Rathinam, Periyakali Saravana Bhavan, Gopalan Rajkumar, Virumandi Nathiya, Thangavelu Satgurunathan, Thangaraj Manjula. (2017). Phytochemical Characterization of Alternanthera sessilis and Assessment of its Growth Promoting Potential on the Freshwater Prawn Macrobrachium rosenbergii. International Journal of Research Studies in Zoology. Volume 3, Issue 4, Page No: 25-38.

[28] Kareem, Z. H., Abdelhadi, Y. M., Christianus, A., Karim, M., & Romano, N. (2016). Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (Oreochromis niloticus) and their resistance to Streptococcus agalactiae infection. Fish Physiology and Biochemistry, 42(2), 757–769.

[29] Khalaji Saeed , Mojtaba Zaghari, Somaye Nezafati, (2011). The effects of mannan-oligosaccharides on cecal microbial populations, blood parameters, immune response and performance of broiler chicks under. African Journal of Biochemistry Research Vol. 5(5), pp. 160-164.

[30] Mohammadi, G., Rafiee, G., El Basuini, M. F., Van Doan, H., Ahlfors, H., & Maita, M. (2014). Use of the essential oil of Artemisia nilagirica on the growth and gonadal maturity of Nile tilapia (Oreochromis niloticus) and their resistance to Streptococcus iniae infection. Ind. Crops Prod. 139:111-472.

[31] Muanda, N.F., Dicko, A., Soulimani, R. (2010) Chemical composition and biological activities of Ficus capensis leaves extracts. J. Nat. Prod. 3:147–160.

[32] Negi P.S. (2012), Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application, International journal of food microbiology, 156(1), pp.7–17.

[33] Pillai, P. K. Premalatha, S. and Rajamony, S. (2002). Azolla-A sustainable feed substitute for livestock. Leisa India, 4(1): 15-17.

[34] Pitcher, T.J. and Hart ,P,J.B.(1982). Fisheries Ecology. London and Canderra;Crom Helm Ltd.(American edition):  The Avi pubi Co.,Linc. Westport,Conn.414 pp.

[35] Prabina, B.J. Kumar, K. (2010). Dried Azolla as a nutritionally rich cost effective and immuno-modulatory feed supplement for broilers. Asian J. Anim. Sci. 5, 20–22.

[36] Quezada-Rodríguez, D. R. P., & Fajer-Ávila, E. J. (2016). The dietary effect of ulvan from Ulva clathrata on hematological-immunological parameters and growth of tilapia (Oreochromis niloticus). Journal of Applied Phycology, 1–9.

[37] Rajkumar, G., Bhavan, P. S., Srinivasan, V., Asaikuttu, A., Udayasuriyan, R., Karthik, M., & Satgurunathan, T. (2018). Effect of Marine Alga (Turbinaria ornata) Mixed Diet on Some Aspects of Biology of Post Larval Macrobrachium Rosenbergii. Proceedings of the Zoological Society.

[38] Setiawati, M., Jusadi, D., Laheng, S., Suprayudi, M. A., and Vinayam, A. (2016a). The enhancement of growth performance and feed efficiency of Asian catfish, Pangasianodon hypophthalmus fed on Cinnamomum burmannii leaf powder and extract as nutritional supplementation. Aquaculture, Aquarium, Conservation and Legislation, 9(6), 1301-1309.

[39] Shimoyamada, M., S. Kudo, K. Okubo, F. Yamauchi and K. Harada, (1990). Distributions of saponin constituents in some varieties of soybean plant. Agric. Biol. Chem., 54: 77-81.

[40] Smith, R. G. (1971) . A method for measuring digestibility and metabolizable of energy of feeds . Prog. Fish Cult. . 33 : 132 -134.
[41] Sreenath, Bhaskaran Kunnathupara, S. Sundaram, Velliyur Kanniappan Gopalakrishnan, Poornima Kannappan. (2016). Quantitative phytochemical analysis, In vitro antioxidant potential and gas chromatography-mass spectrometry studies in ethanolic extract of Azolla microphylla. Clinical Research 9(2), p:318-323.

[42] Sultanbawa Y. (2011), Plant antimicrobials in food applications: Minireview. Science against microbial pathogens: communicating current research and technological advances, 1084–1092.

[43] Thangaraj Manjula, Saravana Bhavan P, Rajkumar G, Muralisankar T, Udayasuriyan R, Kalpana R. (2018), Phytochemical Characterization of Eichhornia crassipes and Sargassum cristaefolium, and Their Effects on the Growth of the Prawn Macrobrachium rosenbergii. Scholars Academic Journal of Biosciences.

[44] Wee, K. L. and Shu, S. W. (1989). The nutritive value of boiled full-fat soybean meal in pelleted feed for Nile tilapia. Aquaculture, 81: 303-314.

[45] Yang, X., Guo, J. L., Ye, J. Y., Zhang, Y. X., & Wang, W. (2015). The effects of Ficus carica polysaccharide on immune response and expression of some immune-related genes in grass carp, Ctenopharyngodon idella. Fish & Shellfish Immunology, 42(1), 132–137.

[46] Yigit Demet, (2018). Antimicrobial and Antioxidant Evaluation of Fruit Extract from Cornus mas L.

[47] Yilmaz, S. (2019a). Effects of dietary blackberry syrup supplement on growth performance, antioxidant, and immunological responses, and resistance of Nile tilapia, Oreochromis niloticus to Plesiomonas shigelloides. Fish & Shellfish Immunology, 84, 1125–1133.

[48] Zahran, E., Risha, E., Abdelhamid, F., & Allah, H. (2014). Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (Oreochromis niloticus). Fish & Shellfish Immunology. 38(1), 149–157.

[49] Zamuzzo, F. S., Urbinati, E. C., Rise, M. L., Hall, J. R., Nash, G. W., & Gamperl, A. K. (2015). Aeromonas salmonicida induced immune gene expression in aloe vera fed steelhead trout, Oncorhynchus mykiss (Walbaum). Aquaculture, 435, 1–9.