Plant Archives Volume 20 No. 2, 2020 pp. 5177-5185 e-ISSN:2581-6063 (online), ISSN:0972-5210

EFFECT OF USE OF THE *AZOLLA* PLANT INSTEAD PARTIAL FOR SOYBEAN MEAL IN THE RATION OF COMMON CARP FISH *CYPRINUS CARPIO* L. IN SOME PRODUCTION STANDARDS

Ammar A. Abd-Alnafi Al-Aaraji* and Nidhal T. Taha

Department of Animal Production, College of Agriculture and Forestry, Mosul University, Iraq.

Abstract

The soybean meal is an important source of protein in the feed ration of the common carp fish *Cyprinus carpio* L. and requires its import from outside the country which increases the cost of the dietary ration of fish. The aim of this study is to use *Azolla sp.* As a source of protein it is a partial substitute for soybean meal to reduce the cost of feeding the fish. The experiment included feeding carp fish using five experimental diets containing *Azolla* in percentages (0, 2.5%, 5%, 7.5%, 10%) of the total ration and *Azolla* substitutions (0, 15%, 30%, 45%, 60%) an alternative to soybean meal, and the use of glass ponds in a 56-day carp growth experiment. The results of the statistical analysis of the weight gain values (g/fish) showed that there were significant differences ($P \leq 0.05$) for the experimental treatments and the second treatment (*Azolla* 2.5%) was high significance compared to the rest of the treatments, while the values of the growth rate and the relative growth rate% and specific growth rate to There were no significant differences in the treatments (1, 2, 3, 4) respectively and differed significantly with the fifth treatment. The results of the statistical analysis in the values of the feed conversion ratio and the percentage of feed efficiency ratio% indicated that there were no significant differences between the second treatment (*Azolla* 2.5%) and the first treatment (control) and the presence of significant differences ($P \leq 0.05$) with the rest of the treatments. The results of the statistical analysis of the precipitated protein values, the protein efficiency ratio and the protein productive value% confirmed that there were no significant differences for the treatments (1, 2, 3) respectively and differed significantly ($P \leq 0.05$) with the fourth and fifth treatment. It is inferred that the *Azolla* can be substituted as a partial substitute for soybean meal (15%) as the best growth criterion.

Key words: *Azolla*, Soybean meal, Ration, *Cyprinus carpio* L.

Introduction

Fish is an inexpensive food for the consumer with a high nutritional value because it is rich in proteins, unsaturated fatty acids, phosphorus and calcium (Dallinger *et al.,* 1987) and after increasing health awareness of the value of fish in nutrition and increasing population and overfishing of fish and marine pollution led to increased demand for fish for the purpose of dam The local market needs of fish and the search for alternatives to fish production. Hence the development of fish farming started by raising fish especially the common carp *Cyprinus carpio* L. which is the most common fish species living in freshwater bodies lakes dams and streams (Vilizzi *et al.,* 2015). The growth of a good carp fish and the ability to withstand the harsh environment and its adaptation to industrial feeds made it enter in many countries of the world (Khan *et al.,* 2016), its ability to accept and use grains in its nutrition and rapid daily growth reaches up to (2-4%) of body weight, The fish weighs (0.6 - 1.0) kg during one season in the ponds. Feeding fish accounts for more than 50% of the cost of cultivation and requires finding substitutes for expensive feed materials such as soybean meal with plant sources of protein that are inexpensive. One of these alternatives is *Azolla sp.* (Sajid *et al.,* 2016).

Azolla contains about 19-30% raw protein on a dry weight basis when conditions are appropriate for growth (Peters *et al.,* 1979 and Becking, 1979), and also contains vitamin A, vitamin B_{12} and beta-carotene which stimulate fish growth (Pillai *et al.,* 2002) The research involved replacing *Azolla* as an alternative to soybean meal.

The study aimed to find out the effect of using the *Azolla* plant in different proportions partly as a substitute

***Author for correspondence :** E-mail : ammar.ali.agha@gmail.com

for soybean meal in experimental carp ration on some production criteria.

Materials and Methods

The study was conducted in the fish laboratory of the College of Agriculture and Forestry, University of Mosul for a period of ten 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, with feeding all AUTOSAN type air compressor tubs of Chinese origin.

150 fish were distributed at an average weight of 8.72 ± 0.05 g/fish in 15 glass basins with ten fishes/ponds, and with three replicates/treatment, the fish remained in the ponds for two weeks to adapt them to the laboratory and pond environment and learn to eat food.

These basins were equipped with liquid water from a large water tank inside the laboratory in which the water was stored for 24 hours to ensure that it is free of chlorine and obtain water at a moderate temperature. The laboratory temperature was controlled between (25-30) Celsius using air conditioners.

It disposes of waste excreted and food residues in ponds on a daily basis by partial substitution of siphon water by 20-25% and supplementing water from the tank, the fish are fed twice daily during the adaptation period until the start of the research experiment.

The water temperature of the glass ponds was measured by a mercury thermometer and was at a temperature of 25°C It was the appropriate temperature for common carp growth (Crockford and Johnston, 1990). Dissolved oxygen was measured in ponds using an EXTECH D0600 field device at a rate of 5.5 mg/l, and the pH level was 7-7.3 using the LABTECH (DIGITAL pH METER) device. It is within the recommended limits (FAO, 1981).

The five experimental rations in the fish laboratory were made from feed materials that were ground by a German origin laboratory mill, by replacing the *Azolla* plant in part as a substitute for soybean meal in different proportions (0%, 2.5%, 5%, 7.5%, 10%) for treatments (1, 2 , 3, 4, 5) respectively, 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 and a cup of warm water was added to the mixture and then the mixture was placed in the national meat grinding machine (Japanese origin with 4 mm holes) gave small and coherent prints and dried for three days cut into small pieces Fits the size of the mouth of the experimental fish and placed in opaque bags to prevent exposure to light and kept in plastic boxes. The replacement process was performed as follows:

1- Control ration (1): free from *Azolla.*

2- Ration (2): It contains *Azolla* 4.5% and soybean meal 27.5% of total ration, that is a substitution rate 15% of the soybean meal.

3- Ration (3): It contains *Azolla* 9% and soybean meal 25% of total ration, that is a substitution rate 30% of the soybean meal.

4- Ration (4): It contains *Azolla* 13.5% and soybean meal 22.5% of total ration, that is a substitution rate 45% of the soybean meal.

5- Ration (5): It contains *Azolla* 18% and soybean meal 20% of total ration, that is a substitution rate 60% of the soybean meal.

Table 1: Composition of experimental rations components (%) resulting from substituting different proportions of *Azolla* as a partial substitute for soybean meal.

Experimental rations Ingredients used	(1) *Azolla* (zero) control	(2) *Azolla* by substitution 15%	(3) *Azolla* by substitution 30%	(4) *Azolla* by substitution 45%	(5) *Azolla* by substitution 60%
Fish meal	10	10	10	10	10
Soybean meal	30	27.5	25	22.5	20
*Azolla*m	—	4.5	9	13.5	18
Barley	20	20	20	20	20
Yellow corn	18.5	16.5	14.5	12.5	10.5
Bran	19	19	19	19	19
Salt	0.5	0.5	0.5	0.5	0.5
Vitamins	1	1	1	1	1
Limestone	0.5	0.5	0.5	0.5	0.5
Bonding article	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Experiment fish were fed on experimental rations mentioned in table 1 at a rate of 3-5% of body weight, two-meal ration per day was adopted and the amount of ration provided to experiment fish was increased based on the weight gain of fish growth, with fish weights taken every two weeks using electronic sensitive balance (0.01)g Chinese type citizen for eight weeks, and the feed provided to the fish was cut one day per week to increase the fish's appetite to eat the feed for the next day during the trial period.

Methods for measuring the growth criteria for experiment fish:

The criteria for measuring fish growth

calculations were adopted to show the effect of Azolla plant substitutes in part on soybean meal in their growth represented by the total weight gain of fish (T.W.G) (Pitcher and Hart, 1982) and the growth rate of fish Growth Rate (G.R) (Pitcher and Hart, 1982), Relative Growth Rate (R.G.R)% (Uten, 1978), Specific Growth Rate (S.G.R) (Jobling and Koskela, 1996) and Feed Conversion Ratio (F.C.R) (Uten, 1978), Feed Efficiency Ratio (F.E.R) (Uten, 1978), Protein Efficiency Ratio (P.E.R) (Gerking, 1971), Protein Intake and Protein Productive Value (P.P.V) (Jobling and Koskela, 1996), according to the equations:

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

Daily weight gain (g/fish) =

$$\frac{Final\ weight - Initial\ weight}{Number\ of\ days}$$

Daily growth rate (g/fish/day) =

$$\frac{Weight\ gain\,(g)}{Duration\ of\ experiment\,(days)}$$

Relative growth rate (%) =

$$\frac{Final\ weight\ (g) - Initial\ weight(g)}{Initial\ weight\ (g\,/\,fish)} \times 100$$

Specific growth rate =

$$\frac{Log\ final\ weight(g) - Log\ initial\ weight\ (g)}{Duration\ of\ experiment\,(days)}$$

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

Feed efficiency ratio (%) =

$$\frac{Total\ weight\ gain\,(g)}{Feed\ consumption\,(g)} \times 100$$

Protein efficiency ratio = $\frac{Total\ weight\ gain\,(g)}{Protein\ intake\,(g)}$

Protein productive value (%) =

$$\frac{Protein\ gain\,(g)}{Protein\ intake\,(g)} \times 100$$

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

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: (components of nutrients):

Determination of nutrient components (protein, fat, ash, moisture, and nitrogen free extract) in the dry matter was performed:

1- Ingredients of experimental processed fish rations shown in table 2.

2- The eaten part of the fish of the experiment research.

Table 2: The chemical composition (%) of the five experimental rations calculated on the basis of the dry weight in which the protein ratio was established between (26.38-28.13) for the purpose of showing the effect of *Azolla* on the growth of experimental fish.

Experimental rations Ingredients used	(1) *Azolla* (zero) control	(2) *Azolla* by substitution 15%	(3) *Azolla* by substitution 30%	(4) *Azolla* by substitution 45%	(5) *Azolla* by substitution 60%
Humidity	7.75	6.56	7.05	6.55	6.91
Ash	5.68	6.39	6.94	7.77	7.99
Raw fat	6.26	7.53	6.69	6.39	6.92
Raw protein	26.38	25.68	26.38	27.13	28.13
Nitrogen free extract(NFE)	53.93	53.84	52.94	52.16	50.05
Total	100	100	100	100	100
*Metabolicm energy (MJ/kg)	14.49	14.78	14.51	14.44	14.51

*Metabolic energy was calculated based on Smith, (1971) equation: ME(MJ/Kg) = Protein × 18.8 + Fat × 33.5 + NFE × 13.8.

The nutrients in fish rations and body were estimated on a dry weight basis according to the standard methods approved (AOAC, 2000) in estimating the protein, fat, ash and moisture, according to the nitrogen free extract mathematically using the difference method as shown in (Wee and Shu, 1989) 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 and growth rate

The results of the statistical analysis of the final weight rate (g/fish) in table 3 showed that there were no significant differences ($P \leq 0.05$) between the treatments (1, 2, 3, 4) respectively, while a significant difference was observed for the fifth treatment (*Azolla* 10%) it reached (15.07) g/fish. The results of the statistical analysis of the weight gain (g/fish) showed that there were significant differences ($P \leq 0.05$) for the experimental treatments and the second treatment (*Azolla* 2.5%) whose value was (8.44) g/fish with high significance compared to the rest of the treatments, where they differed significantly with The third treatment (*Azolla* 5%) and its value was (7.85) g/fish, and the difference was highly significant with the fourth treatment (*Azolla* 7.5%) and the fifth treatment (*Azolla* 10%) which amounted to (7.77 , 6.20) g/fish respectively, while No significant differences were observed between the third treatment (*Azolla* 5%) and control. The results of the growth rate (g/fish/day) in table 3 showed that there were no significant differences for the four treatments (1, 2, 3, 4) compared to the fifth treatment (*Azolla* 10%) which amounted to (0.11) g/fish/day.

Table 3: Effect of different levels of *Azolla sp.* on the growth criteria, weight gain and growth rate of common carp fed for 56 days (mean ± standard error).

Studied criteria Treatments	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (g/fish)	Growth rate (g/fish)
Control (1)	8.46 ± 0.12a	16.68 ± 0.19a	8.22 ± 0.08ab	0.15 ± 0.01a
Azolla (2.5%) (2)	8.69 ± 0.40a	17.14 ± 0.19a	8.44 ± 0.219a	0.15 ± 0.01a
Azolla (5%) (3)	9.18 ± 0.28a	17.03 ± 0.31a	7.85 ± 0.32ab	0.14 ± 0.01a
Azolla (7.5%) (4)	8.92 ± 0.27a	16.69 ± 0.27a	7.77 ± 0.10b	0.14 ± 0.01a
Azolla (10%) (5)	8.87 ± 0.07a	15.07 ± 0.01a	6.20 ± 0.08c	0.11 ± 0.01b

*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 different levels of Azolla sp. on the criteria for relative growth and specific growth of common carp fed for 56 days (mean ± standard error).

Studied criteria Treatments	Specific growth	Relative growth %
Control (1)	97.22 ± 0.79a	1.21 ± 0.01a
Azolla (2.5%) (2)	97.72 ± 6.57a	1.22 ± 0.01a
Azolla (5%) (3)	85.84 ± 5.12a	1.21 ± 0.01a
Azolla (7.5%) (4)	87.29 ± 3.13a	1.21 ± 0.01a
Azolla (10%) (5)	69.95 ± 1.41b	1.16 ± 0.01b

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

Sahu, (2006) reported that *Azolla* plant is a good source of protein and can be used up to 16.25% by substituting 10% as a substitute for fish meal in the feed rations of *Labeo rohita Fry* fish, and *Azolla* substitution can reach 25% for rohita fish feeding (Datta, 2011).

It was observed in our current research that an increase in the percentage of *Azolla* substitution of 15% in the experimental rations of common carp leads to a decrease in the weight gain rate and growth rate, and this is what Sahu pointed out, (2006), that the decrease in growth due to the increased concentration of *Azolla* in dietary rations is due to unbalance of amino acid composition in *Azolla* protein, *Azolla* values were higher with methionine and lysine amino acids compared to soybean meal and meat and bone powder (Das *et al.,* 2018). Our research agreed with Gangadhar *et al.,* (2015) when adding different levels of *Azolla* in *Labeo fimbriatus* fish, and researcher Cruz-Velásquez *et al.,* (2014) showed similar results to our research, as well as the compatibility of results with Micha *et al.,* (1988) where he mentioned a decrease Fish (*Oreochromis niloticus* and *Tilapia rendalli*) grow when adding *Azolla* to feeding rations. The differences in the criteria for fish growth are also attributed to the different ratios of *Azolla* added to the ration that vary the energy ratios of experimental feeds (Lupatsch, 2001).

Relative and specific growth rate

The results of the statistical analysis in table 4 showed that the relative growth values showed that there were no significant differences for the treatments (1, 2, 3, 4) respectively while a significant difference was observed in the fifth treatment (*Azolla* 10%) compared to the rest of the treatments which amounted to (69.95%). The results of the statistical analysis of the specific growth values indicated that there were no significant differences for the treatments (1, 2, 3, 4) respectively while a significant difference was observed in the fifth treatment (*Azolla* 10%) compared to the rest of the treatments and its value (1.16).

It was observed during the current research that the relative growth rate% and specific growth in feeding rations using 15% *Azolla* and *Azolla* free control rations were more appreciated than other treatments, and this was agreed with Micha *et al.,* (1988, 1989) and El-Sayed, (1992), The researchers observed that when adding *Azolla* to the fish *O. niloticus* feed it reduced the growth rate. This decrease in growth is linked to a decrease in the intake of food by regular carp and trout (Ogino, 1980), and the lack of nutrition reduces the rate of fish growth, Increased *Azolla* reduced the digestion and growth rate in the ration due to the difficulty of digesting raw vegetable ingredients Because the *Azolla* plant contains large quantities of non digestible fibers and is generally considered to be complex molecules represented by cellulose and starch (De Silva *et al.,* 1995), because cellulose is difficult to digest by fish while starch is converted to glucose and in turn saves energy. Fagbenro *et al.,* (2004) and Omoregie *et al.,* (1993) explain that increased fiber content in dietary rations of fish and vegetable sources has a negative effect on weight gain, protein representation and growth response in Nile tilapia, Anderson *et al.,* (1984) noted when the fiber level exceeds 100 g/kg reduced the feeding efficiency and digestion of tilapia fish resulting in poor fish growth. As well as the *Azolla* containing anti-nutritional factors that may adversely affect the efficiency of fish feeding (Krogdahl *et al.,* 2010).

Fasakin, (1999) indicated that the *Azolla africana* plant contains a small fraction of some anti-nutritional factors such as cyanide, tannin and phytic acid. Micha *et al.,* (1998) observed that the growth rate of tilapia fish decreased gradually with the increase in the level of the *Azolla microphylla* plant it is evident from the current research that the growth performance of fish is due to the lack of quality protein, amino acids, fats and energy digestion of the plant the nutritional quality of the *Azolla* plant can be improved by adding chemical and mechanical treatments it concentrates the protein content and removes the effects of anti-nutritional substances but it will increase the cost of feed.

Food intake, feed conversion ratio and feed efficiency ratio

The results of the statistical analysis in table 5 of the amount of food intake (g/fish) showed that there were no significant differences between the different treatments. While the results of the statistical analysis of the feed conversion ratio indicated that there were no significant differences in the first and second treatment (*Azolla* 2.5%) and their values (2.58 , 2.57) respectively, with a highly significant difference ($P \leq 0.05$) for the fifth treatment (*Azolla* 10%) reached Its value (3.38) and this value is high compared to the third treatment (*Azolla* 5%) and the fourth (*Azolla* 7.5%) which differed morally, and we did not notice a significant difference between the third transactions (*Azolla* 5%) and the fourth (*Azolla* 7.5%) whose value was (2.76, 2.91) respectively. The results of the statistical analysis in the values of feed efficiency ratio% showed that there were no significant differences in the first treatment and the second treatment (*Azolla* 2.5%) and differed significantly from the third treatment (*Azolla* 5%) and the fourth (*Azolla* 7.5%), while no significant differences were recorded between the third treatment (*Azolla* 5%) and the fourth (*Azolla* 7.5%), while a highly significant difference ($P \leq 0.05$) was observed between the first and the second treatment (*Azolla* 2.5%) compared to the fifth treatment (*Azolla* 10%) and differed significantly with the third and fourth treatment.

Table 5: Effect of different levels of *Azolla sp.* on the food intake criteria, feed conversion ratio and feed efficiency ratio of common carp fed for 56 days (mean ± standard error).

Studied criteria Treatments	Feed efficiency ratio%	Feed conversionratio	Food intake (g/fish)
Control (1)	21.16 ± 1.15a	2.58 ± 0.15b	39.10 ± 2.19a
Azolla (2.5%) (2)	21.62 ± 1.18a	2.57 ± 0.21b	39.37 ± 3.02a
Azolla (5%) (3)	21.58 ± 0.60a	2.76 ± 0.17ab	36.51 ± 2.33ab
Azolla (7.5%) (4)	22.60 ± 0.65a	2.91 ± 0.10ab	34.45 ± 1.25ab
Azolla (10%) (5)	20.91 ± 1.46a	3.38 ± 0.28a	29.98 ± 2.33b

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

The results of the feed conversion ratio announced by Fiogbé *et al.,* (2004) indicated that the observed growth difference was due to insufficient levels of amino acids and caused a reduction in food intake, protein synthesis and growth. This was confirmed by Cowey (1992), Cole and van Lunen (1994).

The researchers observed Almazan *et al.,* (1986) with *O. niloticus*, Antoine *et al.,* (1986) with *O. niloticus*, *Cichlasoma melanurum* and Micha *et al.,* (1988) with *O. niloticus* and *T. rendalli* and Joseph *et al.,* (1994) with *Etroplus suratensis* fish Decreased growth performance and feed conversion ratio when increasing *Azolla* supplementation in nutrition. A similar decrease in growth rate and feed conversion ratio was observed in fish feeding on high levels of *Azolla* concentration and it was found that the lower growth was due to an imbalance in the composition of the amino acid of the Azolla protein (Sahu and Sheeno, 2006).

Almazan *et al.,* (1986) indicated that the *Azolla pinnata* protein is limited to a small amount of tryptophan amino acid and a severe deficiency of threonine amino acid, causing confusion in increasing feed consumption for the purpose of compensating for the deficiency of amino acids when increasing the *Azolla* concentration in feed rations.

The reason for the increase in the feed conversion ratio when increasing the concentration of *Azolla* compared to the feed rations free from *Azolla* is due to the presence of high dietary fiber and ash content, where the highest percentage of the feed conversion ratio of Nile tilapia fish (4.2) which was fed on food rations fortified with dried *Azolla* (*A. pinnata*) at a level of 25% (Abdel-Tawwab, 2008), a decline in feed digestion and consumption occurs at the inclusion of high levels of *Azolla* by affecting the activity of digestive system enzymes and thus growth performance is linked to improved nutrient digestion and nutritional efficiency (Hong *et al.,* 2004, Sithara *et al.,* 2008).

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

The results of the statistical analysis in table 6 for the values of the protein intake (g/fish) showed no significant differences between the different treatments. While the results of the statistical analysis of the protein efficiency ratio values indicated that there were no significant differences for the treatments (1, 2, 3) respectively while a significant difference (P≤0.05) was observed for the treatments (1, 2, 3) compared to the fifth treatment (*Azolla* 10%) they differed significantly with the fourth treatment (*Azolla* 7.5%) which amounted to (1.27). The results of the statistical analysis of the precipitated protein values confirmed that there were no significant differences for the first, second and third treatment respectively while a significant difference with the fourth treatment (*Azolla* 7.5%) and highly significant difference (P≤0.05) with the fifth treatment (*Azolla* 10%) appeared and the highest value for the precipitated protein was for the second treatment (*Azolla* 2.5%) and reached (1.42%), while the lowest value for the precipitated protein is for the fifth treatment (*Azolla* 10%) and it reached (1.15%). The results of the statistical analysis of the criteria for the protein productive value % showed that there were no significant differences for the first three treatments respectively and differed significantly with the fourth treatment (*Azolla* 7.5%) and the fifth treatment (*Azolla* 10%), no significant difference was observed between the fourth treatment (*Azolla* 7.5%) and the fifth (*Azolla* 10%). The highest protein productive value for the second treatment (25.78%) and the lowest protein productive value was for the fifth treatment (19.67%).

Table 6: Effect of different levels of *Azolla sp.* on the criteria of the protein intake, protein efficiency ratio, precipitated protein, and protein productive value of common carp fed for 56 days (mean ± standard error).

Studied criteria Treatments	Protein intake (g/fish)	Protein efficiency ratio	Precipi-tated protein	Protein productive value %
Control (1)	5.58 ± 0.30a	1.48 ± 0.08a	1.40 ± 0.01a	25.26 ± 1.16a
Azolla (2.5%) (2)	5.55 ± 0.30a	1.53 ± 0.12a	1.42 ± 0.04a	25.78 ± 1.40a
Azolla (5%) (3)	5.69 ± 0.16a	1.39 ± 0.09a	1.40 ± 0.02a	24.68 ± 0.51a
Azolla (7.5%) (4)	6.13 ± 0.18a	1.27 ± 0.05ab	1.30 ± 0.01b	21.30 ± 0.66b
Azolla (10%) (5)	5.88 ± 0.41a	1.07 ± 0.08b	1.15 ± 0.01c	19.67 ± 1.20b

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

The significant decrease in protein productive value %, precipitated protein and protein efficiency ratio indicates an increase in the level of *Azolla* our current research agreed with Micha *et al.,* (1988) and El-Sayed, (1992). (El-Sayed, 1992) explained that the lowest percentage can be replaced by *Azolla* 25% as a substitute for fish meal in the ration, because adding *Azolla* to fish feed rations depends on the cost / benefit ratio, taking into account the differences between the types of fish, their ages and their ability to digest Plant material and the nature of fish feed whether plant or animal or mixed (Buddington, 1979 and Caulton, 1978).

Increasing the concentration of *Azolla* with a decrease associated with the soybean meal leads to a lack of essential amino acids and consequently a decrease in protein quality, as Santiago *et al.,* (1988) and Olvera-Novoa *et al.,* (1990) indicate that this phenomenon has found similar results in the use of *alfalfa* and *Azolla pinnata* in nutritional ration for tilapia. Fasakin and Balogun (1998) explained when precipitated protein and protein efficiency ratio decreased as the level of *Azolla* in the feeding ration of *Clarias gariepinus* increased. Our current research agreed with Hossain *et al.,* (2002) and Fasakin *et al.,* (2001) replacing fish meal with vegetable protein in the dietary rations of Nile tilapia.

The high fiber content speeds food through the intestines of animals (Bender, 1967) which reduces the time available to digest and absorb nutrients. There is a negative relationship between the activity of the protease enzyme and the concentration of the Azolla protein for feed, it is mentioned when changes occur in the activity of protein hydrolysis enzymes in common carp fish that depend on the composition of dietary rations, as the *Azolla*-free food may be more adapted to the protein hydrolysis enzyme which gradually deteriorates due to increased Azolla. Protein degradation activity may have decreased due to trypsin inhibitors present in the vegetable tissues of many aquatic plants (Gleen *et al.,* 1982 and Yousif *et al.,* 1994) as trypsin inhibitors impair protein digestion and absorption (Olvera-Novoa *et al.,* 1990).

Chemical composition of fish body

The results of the statistical analysis of the chemical composition of the fish body table 7 of the values of humidity percentage for the components of the eaten part of the fish indicated that there were significant differences

Table 7: Effect of different levels of *Azolla sp.* on the chemical composition of the common carp fish body (mean ± standard error).

Treatments Studied criteria	The chemical composition of fish body% after feeding it on different experimental rations					
	Before the experiment	Control (zero) (1)	*Azolla* (2.5%) (2)	*Azolla* (5%) (3)	*Azolla* (7.5%) (4)	*Azolla* (10%) (5)
Humidity	abc72.17± 0.13	bc72.07± 0.35	c72.01± 0.05	a72.50± 0.16	ab72.43± 0.12	abc72.28± 0.01
Dry matter	abc27.83± 0.13	ab27.93± 0.11	a27.99± 0.05	c27.50± 0.16	bc27.57± 0.12	abc27.72± 0.10
Crude protein	c15.32± 0.09	b16.23± 0.07	b16.27± 0.04	ab16.47± 0.12	ab16.53± 0.12	a16.63± 0.16
Raw fat	a7.11±0.08	ab6.81±0.15	abc6.74±0.05	bc6.64±0.24	cd6.38±0.08	d6.22±0.05
Ash	ab3.82±0.17	b3.71±0.13	ab3.86±0.01	c3.32±0.18	bc3.67±0.01	a4.12±0.04
Carbohydrates	a1.58±0.05	b1.18±0.22	b1.12±0.03	bc1.07±0.02	bc0.99±0.07	c0.75±0.15

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

(P≤0.05) between the different treatments where the percentage of humidity in the third treatment (*Azolla* 5%) reached (72.50) from the humidity content of the fish body prior to the first trial and treatment (control) was (72.17, 72.07) respectively. The results of the statistical analysis of the percentage values of the dry matter percentage of the components of the eaten part of the fish showed that there were significant differences (P≤0.05) between the different treatments and the highest value of the second treatment (*Azolla* 2.5%) and reached (27.99). The results of the statistical analysis showed the values of the crude protein ratio to the presence of highly significant (P≤0.05) for the fifth treatment (*Azolla* 10%) and its value (16.63) for the ratio of crude protein before the experiment and reached (15.32) and the first treatment amounted to (16.23), while it was not recorded significant differences between the second treatment (*Azolla* 2.5%) and the first treatment. Likewise, no significant difference was recorded between the fourth treatment (*Azolla* 7.5%) and the third treatment (*Azolla* 5%). The results of the statistical analysis of the raw fat percentage values indicated the presence of significant differences (P≤0.05) among the different treatments, significant differences were observed (P≤0.05) for the ash percentage values between the different treatments and the highest ash ratio value was in the fifth treatment (*Azolla* 10%) and its value was (4.12) and the lowest value was for the third treatment (*Azolla* 5%) and reached (3.32). From the table 7 in which the results of the statistical analysis of the carbohydrate percentage values were found there were significant differences (P≤0.05) between the different treatments and no significant differences were observed between the first treatment (control) and the second treatment (*Azolla* 2.5%) as well as the absence of significant differences between the third treatment (*Azolla* 5%) and the fourth treatment (*Azolla* 7.5%).

Antoine *et al.,* (1986) indicated when feeding *O. niloticus* and *C. melanurum* fish on Azolla they had high humidity content and low fat concentrations in the fish body. El-Sayed, (1992) explained that body protein and fat were negatively associated with high levels of *Azolla* in fish rations while the association was positive for ash and body humidity. Raw body protein data in our current study contrasted with the illustration he mentioned (El-Sayed, 1992) but by agreement regarding the fat, ash and body humidity content (Micha *et al.,* 1988) that the addition of *Azolla* at increased levels in fish rations of *O. niloticus* and *T. rendalli* resulted in a reduction in the percentage of fats in the body tissues of both species and no effect on the crude protein content.

Das, (2018) stated when adding different levels of *Azolla* to the Thai silver *Barbonymus gonionotus* feed ration that raw fat, protein and ash were negatively correlated while the correlation was positive relative to humidity.

Conclusion

1- *Azolla* is a good source of protein and can be replaced in rations for common carp fish by 15% partly as a substitute for soybean meal without any harmful effect.

2- Adding *Azolla* to fish rations reduced the percentage of fat in fish muscles.

3- *Azolla* is an inexpensive protein source compared to other protein sources that require import.

4- *Azolla* plant productivity is characterized by its high rate of growth and its tolerance to harsh environmental conditions.

Acknowledgments

I would like to express my deepest gratitude and supervisor ''Dr. Nidhal Tahseen Taha'' for his helper advices, meticulous supervision, and guidance throughout the execution of this research.

My special thanks and gratitude to my parent and their continued support in life, my beloved wife, all members of my (University of Mosul, Collage of Agriculture and Forestry) and all my friends.

References

A.O.A.C. Association of official analytical chemists (2000). 17 th ed. V11. USA.

Abdel-Tawwab M. (2008). The preference of the omnivorous-macrophagous. *Tilapia zillii*.

Gervais. to consume a natural free-floating fern. *Azolla pinnata*. *Journal of the World Aquaculture Society,* **39(1):** 104-112.

Almazan, G.J., R.S.V. Pullin, A.F. Angels, T.A. Manolo, R.A. Agbayani and M.T.B. Trono (1986). *Azolla pinnata* as dietary component for *Nile tilapia*, *Oreochromis niloticus*. In J.L. Maclean, L.B. Dizon and L.V. Hosillos Eds., The first Asian fisheries forum proceedings pp: 523-528. Manila: Asian Fisheries Society.

Anderson, J., A.J. Jackson, A.J. Matty and B.S. Capper (1984). Effects of dietary carbohydrate and fiber on the Tilapia *Oreochromis niloticus* L. *Aquaculture,* **37:** 303-314.

Antoine, T., S. Carraro, J.C. Micha and C. Van Hove (1986). Comparative appetency for *Azolla* of *Cichlasoma* and *Oreochromis tilapia*. *Aquaculture,* **53:** 95-99.

Becking, J.H. (1979). Environmental requirements of *Azolla* for use in tropical rice production. In: Nitrogen and Rice. International Rice Research Institute, Los Banos, Laguna, Philippines. pp: 345-374.

Bender, A.E. (1967). Dietetic Foods. Leonard Hill Books, London, pp: 211.

Buddington, R.K. (1979). Digestion of an aquatic macrophyte by *Tilapia zillii* Gervais. *J. Fish. Biol.,* **15:** 449-455.

Caulton, M.S. (1978). Tissue depletion and energy utilization during routine metabolism by sub-adult *Tilapia rendalli* Boulenger. *J. Fish. Biol.,* **13:** 1-6.

Cole, D.J.A. and T.A. Van Lunen (1994). Ideal amino acid patterns. In: Amino Acids in Farm Animal Nutrition. J.P.I. D'Mello (Ed.). The Scottish Agricultural College, Edinburgh, UK, pp: 99-112.

Cowey, C.B. (1992). Nutrition: estimation requirements of rainbow trout. *Aquaculture,* **100:** 177-189.

Crockford, T. and I.A. Johnston (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.

Cruz-Velásquez, Y., C. Kijora, W. Vergara-Hernández and C. Schulz (2014). On-farm evaluation of *Cachama blanca* and *Nile tilapia* fed fermented aquatic plants in a polyculture. *Orinoquia Suplemento,* **18(2):** 269-277.

Dallinger, R., F. Prosi, H. Segner and H. Back (1987). Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia,* **73(1):** 91-98.

Das, M., Rahim, F.I. & Hossain, A.,.2018. Evaluation of Fresh *Azolla pinnata* as a Low-Cost Supplemental Feed for Thai Silver Barb *Barbonymus gonionotus*. *Fishes,* **3(15):** 1-11. doi:10.3390/ûshes3010015.

Datta, S.N. (2011). Culture of *Azolla* and its efficacy in diet of *Labeo rohita*. *Aquaculture*, **310(3-4):** 376-379.

De Silva, S.S. and T.A. Anderson (1995). Fish Nutrition in Aquaculture. Chapman & Hall Aquaculture, London, UK, 319 pp.

Duncan, C.B. (1955). Multiple rang and Multiple " F " test. *Biometric,* **11:** 1-12.

El-Sayed, A.F.M. (1992). Effect of substituting fish meal with *Azolla pinnata* in practical diets for fingerling and adult Nile tilapia, *Oreochromis niloticus* L. *Aquacult. Fish. Manag,* **23:** 167-173.

Fagbenro, O.A., T.T. Akande, T.T. Fapohunda and Y. Akegbejo-Samsons (2004). Comparative assessment of roselle *Hibiscus sabdariffa var.* sabdariffa seed meal and kenaf *Hibiscus sabdariffa var.* altissima seed meal as replacement for soybean meal in practical diets for fingerlings of Nile Tilapia. *Oreochromis niloticus*. Pages 277-288 in R. Bolivar, G. Mair and K. Fitzsimmons. editors. The 6th International Symposium of Tilapia in Aquaculture. Manila. The Philippines; September 14-16. 2004.

Fasakin, A.E. and A.M. Balogun (1998). Evaluation of the dried water fern *Azolla pinnata* as a replacer of soybean dietary components for *Clarias gariepinus* fingerlings. *J. Aquacul. Trop.,* **13:** 57-64.

Fasakin, E.A. (1999). Nutrient quality of leaf protein concentrates produced from water fern *Azolla africana* Desv and duckweed *Spirodela polyrrhiza* L. *Schleiden. Biores. Technol.,* **69:** 185-187.

Fasakin, E.A., A.M. Balogun and O.A. Fagbenro (2001). Evaluation of Sun-Dried Water Fern, *Azolla Africana* and Duckweed, *Spirodela polyrrhiza* in Practical Diets for *Nile tilapia*, *Oreochromis niloticus* Fingerlings. *J. Appl. Aquac.,* **11:** 83-92.

Fiogbé, E.D., J.C. Micha and C. Van Hove (2004). Use of a natural aquatic fern, *Azolla microphylla*, as a main component in food for the omnivorous–phytoplankton *Ophagous tilapia*, *Oreochromis niloticus* L. *Journal of Applied Ichthyology*, **20(6):** 517-520.

Food and Agriculture Organization (FAO) (1981). Report of the symposium on new developments in the utilization of the heated effluents in the circulation system for intensive aquaculture stavanger, 29-30, Rome. Italy.

Gangadhar, B., N. Sridhar, S. Saurabh, C.H. Raghavendra, K.P. Hemaprasanth and P. Jayasankar (2015). Effect of *Azolla* incorporated diets on the growth and survival of *Labeo fimbriatus* during fry-to fingerling rearing. *Cogent Food and Agriculture,* **1:** 1055539.

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

Gleen, E.P., M.R. Fontes, M.R. Katzen and L.B. Colvin (1982). Nutritional Value of Halophytes Grown on Hyper Saline Sea Water. In: Biosaline Research: A Look to the Future, Sanpietro, A. (Ed.). Plenum Press, New York, pp: 485-489.

Hong, K.J., C.H. Lee and S.W. Kim (2004). *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food,* **7:** 430-

435.

Hossain, M.A., U. Focken and K. Becker (2002). Nutritional evaluation of dhaincha (*Sesbania aculeata*) seeds as dietary protein source for tilapia *Oreochromis niloticus*. *Aquac. Res.,* **33:** 653-662.

Jobling, M. and R. Koskela (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.

Joseph, A., P.M. Sherief and T. James (1994). Effect of different dietary inclusion levels of *Azolla pinnata* on the growth, food conversion and muscle composition of *Etroplus suratensis* Bloch. *Journal of Aquaculture in the Tropics,* **9:** 87-94.

Khan, M.N., K. Shahzad, A. Chatta, M. Sohail, M. Piria and T. Treer (2016). A review of introduction of common carp *Cyprinus carpio* in Pakistan: Origin, purpose, impact and management. *Croat. J. Fish.,* **74:** 71-80.

Krogdahl, A., M. Penn, J. Thorsen, S. Refstie and A.M. Bakke (2010). Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids. *Aquac. Res.,* **41:** 333-344. livestock.http://www.eriksjodin.net/projects/ theAzollacookingandcultivationproject/ research/Azolla, a sustainable feed for livestock.pdf.

Lupatsch, I., G.W. Kissil, D. Sklan and E. Pfeffer (2001). Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead sea bream *Sparus aurata* L. *Aquaculture Nutrition*, **7(2):** 71-80.

Micha, J.C., T. Antoine, P. Wery and C. Van Hove (1988). Growth, ingestion capacity, comparative appetency and biochemical composition of *Oreochromis niloticus* and *Tilapia rendalli* fed with *Azolla*. In R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Eds.), Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings pp: 347-355. Bangkok: Department of Fisheries.

Micha, J.C., B. N'Guessan and C. Van Hove (1989). The aquatic fern *Azolla* as feed for fish. pp: 677-681. In: *Aquaculture, A Biotechnology in Progress,* **2:** E. de Pauw, E. Jaspers, H. Ackefors and N. Wilkins (Eds). European Aquaculture Society, Bredene, Belgium.

Micha, J.C., T. Antoine, P. Wery, Van Hove and C. Growth (1998). Ingestion capacity, comparative appetency and biochemical composition of *Oreochromis niloticus* and *Tilapia rendalli* fed with *Azolla*. In Proceedings of the Second International Symposium on *Tilapia* in Aquaculture, Department of Fisheries, Bangkok, Thailand, 16-20 March 1987; R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, J.L. Maclean, Eds.; International Centre for Living Aquatic Resource Management: Manila, Philippines.

Ogino, C. (1980). Requirements of carp and rainbow trout for essential amino acids. *Bull. Jpn. Soc. Sci. Fish.,* **46:** 171-174.

Olvera-Novoa, M.A., G.S. Campos, M.G. Sabido and C.A.M. Palacios (1990). The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia *Oreochromis mossambicus*. *Aquaculture,* **90:** 291-302.

Omoregie E. and F.I. Ogbemudia (1993). Effect of substituting fishmeal with palm kernel meal on growth and food utilization of the Nile Tilapia. *Oreochromis niloticus*. *Israeli Journal of Aquaculture – Bamidgeh,* **45:** 113-119.

Peters, G.A., B.C. Mayne, T.B. Ray and R.E. Jr. Toia (1979). Physiology and biochemistry of the *Azolla-Anabaena* symbiosis. In: Nitrogen and Rice. International Rice Research Institute, Los Banos, Laguna, Philippines. pp: 325-344.

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

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

Sahu, T.S.P. (2006). Use of freshwater aquatic plants as a substitute of fishmeal in the diet of *Labeo rohita* fry. *Journal of Fisheries and Aquatic Science*, **1(2):** 126-135.

Sajid, M., K. Noor, J.I. Khalid, A. Muhammad and K. Anjum (2016). Evaluation of water hyacinth *Eichhornia crassipes* supplemented diets on the growth, digestibility and histology of grass carp *Ctenopharyngodon idella* fingerlings. *J. Appl. Anim. Res.,* **46(1):** 24-28.

Santiago, C.B., M.B. Aldaba, O.S. Reyes and M.A. Laron (1988). Response of Nile Tilapia *Oreochromis niloticus* Fry to Diets Containing *Azolla* Meal. Proceedings of 2nd International Symposium on Tilapia Aquaculture, Mar. 16-20, Bangkok,Thailand, pp: 377-382.

Sheeno, T.P. and N.P. Sahu (2006). Use of freshwater aquatic plants as a substitute of fish meal in the diet of *Labeo rohita* fry. *Journal of Fisheries and Aquatic Science,* **1:** 126-135.

Sithara, K. and K. Kamalaveni (2008). Formulation of low-cost feed using *Azolla* as a protein supplement and its influence on feed utilization in fishes. *Current Biotica,* **2:** 212-219.

Smith, R.G. (1971). A method for measuring digestibility and metabolizable of energy of feeds. *Prog. Fish Cult.,* **33:** 132-134.

Statistical Package for Social Science (2001). SPSS Version 10, SPSS Inc, U.S.A.

Uten, F. (1978). Standard methods and terminology in fin fish nutrition from: proc. World Sump. On Fin Fish nutrition and fish feed technology. *Hamburg,* 20-23. June 1978. Vol. 11 Berlin.

Vilizzi, L., A.S. Tarkan and G.H. Copp (2015). Experimental evidence from causal criteria analysis for the effects of common carp *Cyprinus carpio* on freshwater ecosystems: A global perspective. *Rev. Fish. Sci. Aquac.,* **13(3):** 253-290.

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

Yousif, O.M., G.A. Alhadrami and M. Pessarsaki (1994). Evaluation of dehydrated alfa and salt bush atriplex leaves in diets for *Tilapia*. *Aquaculture,* **126:** 341-347.