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EFFECT OF USING DIFFERENT LEVELS OF *AZOLLA* AS A SUBSTITUTE FOR SOYBEAN MEAL IN THE PRODUCTION PERFORMANCE OF FISH CARP

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Abstract

Soybeans meal are important sources of proteins, which account for 50% of the cost of fish diet and because of the need to import them from outside the country will increase the cost of the diet. So, it was necessary the use of inexpensive, available sources that can be produced as a protein source to reduce the cost of the diet and the cost of production. The study was conducted at the Fish Laboratory, Faculty of Agriculture, Anbar University, Republic of Iraq, included this study three experiments: The first experiment: Planting *Azolla* in city Al-Baghdadi, Anbar province, Iraq. The first experiment was aimed at growing *Azolla*. The planting period was 9 months. The second experiment: Included Procedure *Azolla* analysis according to A.O.A.C. The results of the analysis were protein43.6%, ash 26.9%, fat 1.27%, moisture 92.47%, fiber 22.66%) and were classified by the Center for Desert Studies - Anbar University, Iraq. The third experiment: Field Experiment: The experiment was conducted at the Fish Laboratory, Faculty of Agriculture, Anbar University, Republic of Iraq. The trial duration (10 weeks) was carried out, where productivity traits were calculated. The results of the statistical analysis of the productivity traits of the basic weight and the percentage of losses showed no statistically significant differences between the coefficients at a significant level (P≤0.05). The results showed that the second treatment (5% *Azolla*) at a significant difference (P≤0.05) was superior to control treatment and treatments (15% *Azolla* and 25% *Azolla*) respectively for final weight, weight gain, daily weight gain, relative growth rate, specific growth rate, feed consumption, feed efficiency and protein efficiency. There were no statistically significant differences between control treatment and 15% and 25% *Azolla* respectively in the same traits. The results showed a significant Improved (P≤0.05) in the feed conversion ratio treatment in favor of the second treatment compared to the control treatment and other treatments. The dietary conversion ratio for the second treatment was 2.13, while the feeding conversion ratio for the control and third and fourth treatments was 2.54, 2.71 and 2.59, respectively. At the same time, there was no significant difference (15% and 25% *Azolla*) compared with control treatment. The first and second treatments of the protein intake were superior compared to the third and fourth treatments. There were also no significant difference between the first and second treatments, while there were no significant difference between the third treatment and the fourth treatment.

Key words : *Azolla*, Production performance, Fish carp.

Introduction

Nutrition is the most expensive item in the fish breeding sector, accounting for more than 50% of the cost of breeding and many of the traditional materials used as raw materials in the manufacture of the diet such as soybean, which became very expensive due to the increase the cost of import and increase demand (Gangadhar *et al*., 2015). Where the cost of basic protein feedstock has increased sharply in recent years relative to global demand (FAO, 2011). Good quality feeds should provide nutritional requirements for good health, optimal growth, optimal return and minimum waste (Nancy Catherine and Amalaranis, 2016). Protein is the most expensive ingredient in fish diet and is also the most important factor affecting the performance of fish growth and the cost of feed (Luo *et al*., 2004). Reducing nutrition costs can be a key factor for aquaculture success. Research (Nancy Catherine and Amalaranis, 2016) shows that local and non-traditional sources can substitute imported and high-priced feeds materials, despite the

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increase in fish feed prices, the prices of fish products have remained constant, which inevitably affects the economic viability of the thousands of small producers who form the backbone of the aquaculture sector (Rana *et al*., 2009). In recent years, the use of high value nutritious aquatic plants as feed ingredients has taken on a new dimension in the production of the required animal protein at low cost (Gangadhar *et al.*, 2015). Hence, the interest in the flora of *Azolla*, the free floating fern *Azolla*, which belongs to the family Azollaceae which is a good source and protein contain almost on all essential amino acids such as iron, calcium, magnesium, potassium, phosphorus, manganese and others, And regardless of reasonable amounts of vitamin precursors beta-carotene and vitamin B12. It was found that it contains probiotics and bio-polymers (Pillai *et al*., 2002).Thus, it seems that *Azolla* potential source of nutrients and have a high nutritional value to a large extent (Hossiny *et al*., 2008). The *Azolla* plant, which grows in cooperation with green algae - blue Anabaena azollae plant, suitable because of the ease of agriculture and productivity of high quality and good nutritional value (Prabina and Kumar, 2010; Singh and Subudhi, 1978). The study aims the effect to use of levels of *Azolla* as a substitute for soybean meal in production performance to fish.

Materials and Methods

The study was conducted in Anbar province, Republic of Iraq. This study included:

The first experiment: Planting *Azolla*

The study was aimed at growing and growing the *Azolla*. Seven earth ponds were equipped in al-Baghdadi city, west of Al-Anbar province, with an area of 7.5 × 4 m, where they were dug 20 cm deep and coated with polyethylene to prevent water leakage and to prevent salinity from damaging the plant. The soil was then placed in the basin and flooded with a height of 15 cm. An industrial umbrella was put in place to reduce the effect of the sun (50% reduction of the sun) and the planting of the initiator in ponds and fertilized every two days. Nutrients were also used as plant stimulants. An insecticide was used to prevent the spread of pests and the water was replaced every 10 days, to ensure that nitrogen was not accumulating with water. After 10 days, the *Azolla* were spread and the basin was fully covered with *Azolla*. After that, one third of the *Azolla* were transferred from the first basins to new basins and the same methods of first agriculture were followed. The harvesting process was carried out for *Azolla* every two weeks by raising the *Azolla* from the ponds, drying it and measuring the moisture content.

The second experiment : Analysis and classification of *Azolla*.

Azolla was classified by the Center for Desert Studies, Anbar University, Republic of Iraq. He conducted *Azolla* analysis was used according to A.O.A.C. (1980) to measure moisture, protein, fat, ash and fiber.

The third experiment : The Field Experiment

The experiment was conducted at the Fish Laboratory, Faculty of Agriculture, Anbar University, Republic of Iraq. The effect of using different levels of *Azolla* as an alternative to soybeans was studied in the productive traits. A total of 120 fish (common fish) (3.5-4 g) were used and fish were raised in a hall of 12 tubs (70 × 40 × 30 cm and 70 l / tub). Ten fish were placed in the Basin one randomly distributed on the experiment transactions by 3 replications treatment. Each basin was equipped with ventilation tubes and thermometers and the water temperature was controlled using Alheitrut for optimal temperature (24°C). All fish were fed at a rate of 5% of the body weight of the fish during the duration of the experiment with three meals a day (9 am - 1 pm - 6 pm). The diet contained raw protein (30.53-30.37%), Energy represented (2754.32-2627.07 k / kg feed), Crude fat (2.083-2.6165%), Crude fiber (3.578-5.78%), Calcium (0.6837-0.9128%), Total phosphorus (0.4171-0.6871%), Lysine (2.0142-15971%), Methionine (0.918-0.8482%), Cysteine (0.4253-0.3042%), Arginine (2.5569-1.7881%), Phosphor available (0.11889-0.19989%) according to NRC (1994) (Table 1). The first treatment: was control without using *Azolla* as a source of protein, second treatment: use 5% of the *Azolla* of feed ingredients, Third treatment: use 15% of the *Azolla* of feed ingredients, Fourth treatment: use 25% of *Azolla* of feed ingredients.

Underwent a period of acclimatization for two weeks, after which the experiment was conducted for 10 weeks. The weights of the fish were taken every two weeks in the form of individual weights throughout the experiment period by scales accurate.

A study productivity traits : A study was conducted Weight Gain, Weight Gain Daily (Utne, 1978), (R. G. R.) Relative Growth Rate (Utne, 1978), (F. C. R.) Feed Conversion Ratio (Utne, 1978), (S.G.R.) Specific Growth Rate (Jobling and Koskela, 1996), Feed efficiency (McCormic *et al*., 1989), (P. E. R) Protein Efficiency Ratio (Gerking, 1971), Protein lntake (Gerking, 1971).

The statistical analysis of the data of this study was carried out using the steps of the general linear model of the SAS program (2001). The effect of the coefficients on the studied traits was determined using the complete randomized design (CRD). To test significant the

Table 1 : Chemical composition and Ingredients (%) of the diet used in the experiment.

Ingredient	Control	Azolla		
	T1(0%)	T2(5%)	T3(15%)	T4(25%)
Animal protein40%	10	10	10	10
Wheat	10	10	9.5	7.5
Barley	5	5	3.5	4.5
Corn	8,5	8,5	8.5	8.5
Soybean 48%	45	40	32	23
Wheat bran	2	2	2	2
Premix	0.1	0.1	0.1	0.1
Azolla	0	5	15	25
Lysine	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Vegetable oil	1.6	1.6	1.6	1.6
Flour	16	16	16	16
Limestone	1.5	1.5	1.5	1.5
Chemical composition of diet materials (NRC, 1994)				
Energy (kcal/kg)	2754.32	2732.47	2682.37	2627.07
% cp	30.5285	30.2785	30.516	30.376
% fat	2.083	2.198	2.4085	2.6165
% fiber	3.578	4.018	4.841	5.78
%calcium	0.6837	0.7282	0.8216	0.9128
% p	0.4171	0.4706	0.5788	0.6871
Lysine	2.0142	1.9152	1.771	1.5971
Methionine	0.918	0.9015	0.8779	0.8482
Cystine	0.4253	0.3983	0.3538	0.3042
Arginine	2.5569	2.3999	2.0967	1.7881
Phosphorus available%	0.11889	0.13494	0.1674	0.19989

* Protein center for fish feed produced by the Dutch company WAFI, and representative energy (Kaluri = 2183.7, 40% crude protein, 5% crude fat, 2.26% raw fiber, 3.53% calcium, 2.65% total phosphorus, 3.83% lysine, 3. methionine 3.7 %, Arginine 2.57%.

** premix: vitamin A (IU1.000.000), vitamin D (IU300.000), vitamin E (mg5.000),Vitamin B1 (mg500), Vitamin B2 (mg400), Vitamin B6 (mg400),Vitamin B12 (mcg1.000), vitamin C (mg2.500), calcium (mg2.000), folic acid (mcg1.000), Methionine (mg3.500), lysine (mg10.000), phytase (U5.000), iron (mg10.000), manganese (mg10.000), zinc (mg15.000), cooper (mg1000).

differences between the studied averages, The Duncan test (1955) was used at a level below 0.05.

Results and Discussion

This thesis was conducted to determine the effect of the use of *Azolla* on the production performance of the fish. The duration of the experiment was 12 months. The results of this experiment were as follows:

***Azolla* production :** *Azolla* was harvested every 15 days at 6 kg per square meter and 75% of the production was taken (4.5 kg). The remaining 25% (1.5 kg) was left for re-culture in the same ponds. Production was calculated on the basis of 75% production:

$4.5 \times (4 \times 7.5) = 135$ kg per basin

Classification and analysis of *Azolla* : The results of the classification carried out at the Center for Desert Studies - Anbar University as follows:

Division: Pteridophyta, Family: Azollaceae, Genus: Azolla Lam., Species: *Azolla filculoides* Lam.

The results of the analysis of *Azolla* given in Table 2.

The productivity traits

Table 3 shows the results of the effect of using different levels of *Azolla* as an alternative to soybean meal in the production traits. No significant differences were observed in the primary weight and percentage of losses between the control treatment and other experimental treatments at a significant level (P00.05). The results showed that the second treatment (5% *Azolla*) at a significant difference ($P \leq 0.05$) was superior to control treatment and treatments (15% *Azolla* and 25% *Azolla*), respectively for final weight, weight gain, daily weight gain, relative growth rate, specific growth rate, feed consumption, feed efficiency and protein efficiency. There were no statistically significant differences between control treatment and treatments 15% and 25% *Azolla* respectively in the same traits. The results showed a significant improvement ($P \leq 0.05$) in the feed conversion ratio treatment in favor of the second treatment compared to the control treatment and other treatments. The dietary conversion ratio for the second treatment was 2.13, while the feeding conversion ratio for the control and third and fourth treatments was 2.54, 2.71 and 2.59, respectively. At the same time, there was no significant difference (15% and 25% *Azolla*) compared with control treatment. The first and second treatments of the protein intake were superior compared to the third and fourth treatments (15% *Azolla* and 25% *Azolla*). The table 3 shows, there were also no significant difference between the first and second treatments, while there were no

Table 2 : Results of *Azolla* analysis.

Chemical analysis of *Azolla*%				
Protein	Fat	Humidity	Ash	Fiber
43.640±0.68	1.27±0.02	92.47±2.21	26.90±0.26	22.66

Table 3 : Effect of using different levels of *Azolla* as a substitute for soybean meal in the production performance.

Traits	Control	*Azolla*			Trait medium	SEM*	Prob.
	T1	T2(5%)	T3(15%)	T4(25%)			
Basic weight G /fish	38.5	38.8	38.8	38.5	38.7	0.385	N.S**
Final weight G / fish	131B	161A	119B	122B	133	7.85	0.0006
Weight Gain G / fish	92.4B	122A	80.9B	83.9B	95.0	7.59	0.0005
Weight gain daily G/fish/day	1.31B	1.75A	1.15B	1.19B	1.35	0.109	0.0005
Relative growth rate G/fish%	2.38B	3.16A	2.08B	2.17B	2.45	0.182	0.0003
Specific growth rate G/day%	0.756B	0.901A	0.697B	0.717B	0.768	0.039	0.0010
Feed consumption G	233B	261A	210B	217B	230	14.0	0.0092
Feed conversion ratio G/fish	2.54A	2.13B	2.71A	2.59A	2.49	0.130	0.0033
Feed efficiency G /fish	39.5B	46.9A	38.3B	38.6B	40.8	1.40	0.0002
Protein intake	74.8A	79.9A	64.4B	66.4B	71.3	3.76	0.0031
Protein efficiency ratio G/fish	1.24B	1.53A	1.25B	1.26B	1.32	0.092	0.0123
The percentage of losses%	0.00	0.00	3.33	6.66	2.50	4.08	N.S

* SEM: Average standard error. ** N.S: non-significant At a significant level (P≤0.05).

significant difference between the third treatment and the fourth treatment. Where he found through a table (3) that the crude fiber content of the diet added *Azolla* was higher than the control where *Azolla* contains the largest amount of fiber. The mineral content and crude protein gradually increased with increased replacement of the basal diet with *Azolla*, Similar to our findings, have been reported crude protein content of the diet *Azolla* in the range (30.51-30.27%).The total Ash content of the *Azolla* obtained in this experiment was 26.9%. Ali and Leeson (1995) reported that the ash value in *Azolla* is very high at 36.12%. However, Aladade and Iyayi (2006) recorded values almost similar to the current study. Can be attributed to the difference in the composition of nutrients from the diet *Azolla* in various studies to differences in response to the strains of *Azolla* appropriate, such as temperature and environmental conditions, Light intensity and soil nutrients that therefore affect morphology of *Azolla* growth and composition. The insignificant differences in the criteria for the growth of fishes receiving the diets listed in *Azolla* can be attributed to differences in energy content from experimental diets (Lupatsch, 2001). Sahu (2006) reported that the concentration of *Azolla* protein is a good source of protein and can be used up to 16.25% by replacing 10% fish meal in the diet of *Labeo rohita* Fry. According to Datta (2011), *Azolla* can be combined to 25% in the diet of Rohitha. Sahu (2006) shows that the decline in the growth may be due to the imbalance in the amino acid composition from *Azolla* protein. The high level of *Azolla* in diets has resulted in reduced growth. These trends came from of variation the remarkable growth and FCR results announced by the (Fiogbé *et al*., 2004). The inadequate levels of amino acids that are indispensable can lead to not eating and growth.

Conclusion and Recommendations

Conclusion

Transactions *Azolla* as an alternative protein for soybean meal gave the best results in terms of final weight ,Weight Gain, Weight Gain Daily, Relative Growth Rate (R. G. R.), Specific Growth Rate (S.G.R.), Feed efficiency, Protein Efficiency Ratio (P. E. R) And also it gave best Feed Conversion Ratio.

Recommendations

We recommend using 15-20% *Azolla* as a protein substitute for soybeans meal.

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