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Effect of protein content on growth parameters of grass carp *Ctenopharyngodon idella* cultured in earthen ponds.

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ABSTRACT

The current experiment was conducted in Basra Governorate in the ponds of the Aquaculture Unit at the Agricultural Research Station-College of Agriculture-University of Basra, located in Al-Haritha District, for the period from 4/3/2020 to 6/12/2020. Three thousand seven hundred sixty grass carp fingerlings, with an average weight of 16 ± 2.75 g and an average total length of 12.1 ± 0.1 cm, were used in the study. These fingerlings were divided into four treatments, each with 2 replicates, and each replicate was placed in one earthen pond of 600 m². Four diets were manufactured with different protein ratios (T1 20, T2 25, T3 30 and T4 35)%. The current experiment aimed to find the optimal proportion of protein in the grass carp diet, which achieves the best growth and lowest cost. The results of the current experiment were as follows: final average weights were (153.5, 167.4, 181.8 and 272.7) g, weights gain were (132.5, 147.6, 162.8 and 255.9) g, daily growth rates were (0.46, 0.52, 0.60 and 0.95) g/day, specific growth rates were (0.71, 0.78, 0.84 and 1.02) %/day and relative growth rates were (18.87, 20.46, 21.35 and 27.05) % for T1, T2, T3 and T4 respectively. T4 was significantly ($P \leq 0.05$) superior to the rest of the three treatments in all previous growth criteria. In contrast, none of these treatments showed significant differences ($P > 0.05$) for the same criteria. The best food conversion rate was for T4, and the worst rate was for T1 and T2.

Keywords: growth parameters, grass carp, *Ctenopharyngodon idella*

INTRODUCTION

Fish are living organisms similar to other animals in their need for protein, vitamins, mineral salts, growth factors, and energy sources necessary for growth, reproduction, and various physiological functions. Artificial feeds constitute more than 60-65% of fish production costs. It depends on the abundance of natural food in the ponds, and industrial feed allows for an increase in fish density, in addition to the fact that their surplus acts as an indirect fertilizer ¹. Food is the elixir of life for living organisms, including fish and its type and quality. It has a key role in the speed of growth, and the feed cost represents 80% of the total costs of fish production and cultivation ². Experiments have shown that grass carp *Ctenopharyngodon idella* not only controls aquatic weeds but also

grows very fast at the same time, and therefore, it is one of the best-cultured fish if its feed is available³. The various types of carp fish are among the main aquaculture fish in Iraq, as they are suitable for the cultural environment. They also belong to the cyprinidae family that most economic Iraqi fish belong to, especially the common carp *Cyprinus carpio* and grass carp⁴. Grass carp have been extruded from this family and attached to the xenocypridinae family by⁵. Grass carp was the first important freshwater cultivated species around the world in 2020, while the second was silver carp, *Hypophthalmichthys molitrix*, the third was Nile tilapia, *Oreochromis niloticus*, and the fourth was common carp⁶. In Iraq, the trend for breeding and cultivation was for common carp.

MATERIALS AND METHODS

1. Study area: The current study was conducted in Basra Governorate in the earthen ponds of the Aquaculture Unit at the Agricultural Research Station - College of Agriculture - University of Basra, located in Al-Hartha District, for the period from 4/3/2020 to 6/12/2020.

2. Study fish: 3760 grass carp fingerlings were used, with an average weight of 16 ± 2.75 g and an average length of 12.1 ± 0.1 cm.

3. Earthen culture ponds: Eight earthen ponds, with an area of 600 m² per pond with an average depth of 2 meters, were used. The ponds were dried before culturing processes. One pond was filled with water two days before the arrival of fish to reduce the presence of natural enemies to a minimum. All fish were placed in this pond and left for three days to acclimate before being divided into eight ponds filled with water later. 15-25% of the pond water is replaced daily, and about 75% of water is replaced during periodic sampling to facilitate catching fish samples.

4. Fish feed: Four commercial pellet diets (diameter of 4-6 mm) were manufactured by the feed plant of the Agricultural Consultant Office belonging to Agriculture College with different ratios of crude protein (20, 25, 30 and 35) % for treatments T1, T2, T3 and T4 respectively by using different feed ingredients (Table, 1). Fish were fed on these diets throughout the cultivation period. The chemical analysis of the different diets was carried out in the Al-Ghadeer Laboratory, located in the center of Babil Governorate, and three replicates were taken from each of the four treatments.

5. Environmental factors: The water temperature of the ponds was measured with the periodic fish sampling during the experiment using a Chinese-made mercury thermometer to the nearest degree Celsius. The salinity and pH of the ponds were also measured by a German device produced by the Yasa company, and the dissolved oxygen was measured using a digital device of the type Lovibond Senso Direct 150.

6. Fish weight measurements: About 10% of the fish were caught randomly by beach trawls approximately every 20 days (sometimes increased according to the available conditions) from each pond and weighed in the farm using an electronic scale to the nearest gram. The feeding ratio ranged between 3-5% of total fish weight, and the daily feed provided to the fish is adjusted according to the new weights. The fish were handily fed three times daily (morning, noon and afternoon) at the same feeding places for each pond.

7. Growth criteria: The following growth criteria were measured at the end of the experiment according to the following equations:

1. Weight Gain Rate(WGR)

WGR (g) = final weight - initial weight

2. Daily Growth Rate (DGR)

$$\text{DGR (g/day)} = (\text{final} - \text{initial weight}) / \text{time}.$$

3. Relative Growth Rate (RGR)

$$\text{RGR (\%)} = (\text{weight gain}/\text{initial weight}) \times 100.$$

4- Specific Growth Rate (SGR)

$$\text{SGR (\%/day)} = [(\text{Ln final weight} - \text{Ln initial weight})/\text{time}] \times 100$$

5-Feed Conversion Rate (FCR)

$$\text{FCR} = \text{consumed feed}/\text{weight gain}$$

8. Statistical Analysis: Statistical software SPSS IBM (23) and Excel 2013 were used to analyze the data.

Ingredients	Ratio%			
	T1	T2	T3	T4
Fish meal	5	15	25	38
Soybean meal	22	22	22	22
Starch	2	2	2	2
Wheat flour	29	29	29	29
Wheat bran	40	30	20	7
The mixture of vitamins and minerals	2	2	2	2

Table 1. The proportions of the main components included in the composition of the experiment diets.

RESULTS

Table (2) shows the measurements of some environmental factors during the experiment. The water temperature was the most variable, and the highest temperature was recorded (38 °C) in August and the lowest (17 °C) in March, while the changes in the pH were limited and ranged between 7.4 in March and 8.7 in August. The salinity of the water in ponds ranged from 3.3 g/L in March to 6.0 g/L in August, while the highest dissolved oxygen concentration was 7.7 mg/L in March, and the lowest concentration was 4.3 mg/L in August.

Table (3) shows the different growth criteria for the treatments (final weight, weight gain, daily growth, specific growth rate, relative growth rate, and feed conversion rate) for grass carp in eight ponds, with the average of each treatment and the statistical analysis of these criteria. The final average weights recorded were (153.5, 167.4, 181.8, and 272.7) g for treatments T1, T2, T3, and T4, respectively. The final weight of T4 was significantly ($P \leq 0.05$) superior to the rest of the three treatments, and none of these treatments showed any significant differences ($P > 0.05$) between the final weight. It was found that the average weight gain of grass carp was (132.5, 147.6, 162.8, and 255.9) g for treatments T1, T2, T3, and T4, respectively. The weight gain of T4 was significantly

($P \leq 0.05$) different from the three treatments, and none of these treatments showed any significant difference ($P > 0.05$) between them.

The daily growth rates recorded were (0.46, 0.52, 0.60, and 0.95) g/day for T1, T2, T3, and T4, respectively. Statistical analysis proved that there were significant differences ($P \leq 0.05$) in the daily growth rate for T4 with the other three treatments, while there were no significant differences ($P > 0.05$) between these three treatments. The specific growth rates of grass carp were (0.71, 0.78, 0.84, and 1.02) %/day for T1, T2, T3, and T4, respectively. Statistical analysis of the results proved significant differences ($P \leq 0.05$) of specific growth rate for T4 with the other three treatments. There were no significant differences ($P > 0.05$) between these three treatments. The relative growth rates of the four treatments, respectively, were (18.87, 20.46, 21.35, and 27.05) %. The relative growth rate of T4 was significantly ($P \leq 0.05$) different from the other three treatments, while there were no significant differences ($P > 0.05$) among these treatments.

The values of the feed conversion rate were 9.32 for T1, 9.10 for T2, 6.47 for treatment T3 and 4.70 for. It is clear from the previous values that the best feed conversion rate was achieved by fishes of T4 and the worst feed conversion rate for fishes of T1 and T2. The results of the statistical analysis of the feed conversion rate showed significant differences ($P \leq 0.05$) between T4 and T3 and between them with T1 and T2. At the same time, there were no significant differences ($P > 0.05$) between treatments T1 and T2.

Sampling date	Environmental factors			
	Water temperature (°C)	pH	salinity (g/l)	Dissolved oxygen (mg/L)
8/3/2020	17	7.4	3.3	7.7
3/4	21	7.8	4.1	7.4
23/4	23	7.9	4.3	7.1
12/5	26	8.1	4.7	6.7
2/6	28	8.2	5.0	6.5
22/6	28	8.4	5.5	6.3
12/7	33	8.5	5.7	6.0
1/8	38	8.7	6.0	4.3
8/9	28	8.3	5.7	6.0

28/9	27	8.1	5.4	6.5
18/10	24	7.6	5.1	7.5
11/11	22	7.7	4.0	7.6
6/12	18	7.5	3.6	7.7

Table 2. Some environmental factors of water during the experiment.

Growth criteria	Earthen ponds							
	T1P1	T1P2	T2P3	T2P4	T3P5	T3P6	T4P7	T4P8
Final weight (g)	147.1	160.0	180.6	154.3	170.8	192.8	257.0	288.5
Average	153.5b		167.4b		181.8b		272.7a	
Weight Gain (g)	125.1	0.49	0.56	0.48	0.56	176.1	240.0	271.8
Average	132.5b		147.6b		162.8b		255.9a	
DGR (g/day)	0.43	0.49	0.56	0.48	0.54	0.66	0.89	1.01
Average	0.46b		0.52b		0.60b		0.95a	
SGR (%/ day)	0.67	0.75	0.79	0.77	0.76	0.92	1.00	1.05
Average	0.71b		0.78b		0.84b		1.02a	
RGR (%)	17.98	19.77	20.62	20.30	19.4	23.30	26.33	27.77
Average	18.87b		20.46b		21.35b		27.05a	
FCR	9.79	8.86	8.91	9.29	6.27	6.68	4.83	4.57
Average	9.32c		9.10c		6.47b		4.70a	

Table 3. Growth criteria of grass carp fed feeds with different protein ratios. Different letters in the same rows show significant differences ($P \leq 0.05$)

DISCUSSION

The environmental factors measured in this study were suitable for the culture of grass carp, except for a few days when the temperature rose to 38°C, and forced to stop the feeding trials in order to avoid stress arising from high temperature and reduce the heat emitted by the fish as a result of feed metabolism. The best growth was achieved in the current experiment at a temperature range of 22-29°C, similar to what ⁷ found that the highest growth rate of grass carp was achieved at degrees 24-29 °C. The growth in the current experiment decreased at temperatures less than 22°C. This may be due to a decrease in the intensity of feeding and a decrease in metabolic rates. ⁸ found the best food intake rate for grass carp at a temperature of 25°C. ⁹ showed that the range required to achieve the best growth is at a temperature of 19.6-27.2 °C and a salinity level of less than 10 g/l. Most previous studies recorded similar results to the current experiment, such as ¹⁰. The current experiment's results differed from those found by ¹¹, recording a better temperature range of 28.5-32.9 °C. The highest salinity concentration was recorded in the current study at the beginning of the eighth month, reaching 6.0 g /l, and the lowest concentration (3.3 g / l) during the third month. ¹² showed that the grass carp fish did not tolerate salt concentrations higher than 10 g/l., especially in the case of direct exposure to these concentrations. At the same time, ¹³ found that young grass carp did not tolerate sudden changes in salinity at 5 g/l.

The nutritional requirements of young grass carp differ from those of adult fish, as juveniles require higher protein content than adults ¹⁴. Determining the specific nutritional needs of grass carp, especially protein, has been a contention among researchers. ¹⁵ concluded that the protein required for grass carp larvae growth is 33%, fingerlings 30%, and adults 25%. ¹⁶ adopted a protein percentage close to some of the parameters of the current study for fingerlings and adults, 42 and 35%, respectively, while ¹⁷ showed that 34.13-38.31% protein is the optimal rate for the growth of juvenile grass carp. ¹⁸ showed that less than 40% is the best protein level for grass carp growth, while ¹⁹ determined 25% as the best protein for grass carp growth and also showed that the percentages 27% and 30% did not lead to an increase in the weight of grass carp and these percentages were close to the percentages of some of the treatments of the current study. ²⁰ found results differ from current results, where they don't notice any significant differences in the growth of fishes fed on diets of 25 and 35% protein ratio.

The results of the current study showed that grass carp fed on a 35% protein diet recorded higher final weight (272.7 g) compared with the other three treatments. The current study recorded a higher final weight in treatments T3 and T4 than was recorded by ²¹ for grass carp fed nearly the same protein ratio diet; this discrepancy may be due to the difference in culture periods in the two studies. ⁹ recorded less final weights (50.35, 54.45, and 36.75 g) compared with the current experiment; this may be due to the difference in the average initial weight for both studies, 6.3 g compared with 16 g, as well as the difference in the season and duration of the two studies.

The results of the current study showed that the highest rate of weight gain (255.9 g) was recorded by grass carp fed a 35% protein diet, while the lowest (132.5 g) was recorded by grass carp fed a 20% protein diet. The current study outperformed ²⁰ in the average weight gain value as it was (1.6, 2.5, and 3.47) g for their three treatments, respectively, and this may be due to the differences in fish ages and the culturing periods of the two studies. ²¹ recorded weight gains (1.92 and 5.37) g, as the fish were fed on a processed ration containing the same percentage of crude protein found in the rations of T3 and T4

in the current experiment, so this large discrepancy between the results of the two studies may be due to the difference in the duration of the two studies and culture methods.

The daily growth rates in the current experiment were (0.46, 0.52, 0.60, and 0.95) g/day for T1, T2, T3, and T4, respectively. ²² recorded a daily growth rate of 0.43 g/day for the hybrid grass carp cultured under laboratory conditions and fed on zooplankton and phytoplankton. The results of the current study differed from those of ²³ who used aquatic and terrestrial plants to feed grass carp for five months, where the daily growth rate ranged between 1.67-2.91 g/day.

Specific growth is one of the criteria used to evaluate fish growth in nutrition research in which different experimental diets are used in terms of the proportions of substances to be tested for their effects on some of the original components of the diet ²⁴. ²⁵ reached a specific growth rate of 14.05%/day, which is higher than what was recorded in the current study, by using three types of live foods to feed grass carp larvae, and this is due primarily to the difference in the initial weight between the two studies and secondly to the high value of the proteins present in live foods. The results of the current experiment also contrasted negatively with those of ¹¹ in the value of the specific growth rate (1.34%/day) for grass carp fed on processed diets. ²⁴ recorded an SGR% of 0.01 %/day, while ¹⁹ recorded an SGR% of 0.28%/ day; these values were less than the values of the current experiment, which may be due to the absence of natural food in their laboratory experiments on grass carp. The current study also recorded a higher SGR than (0.12%/day) in the study of ²⁶ grass carp cultivated in ponds with an area of 1500 m².

The feed conversion rate is one of the important criteria for determining the utility of consumed feed ²⁷. The values of the feed conversion rate were (9.32, 9.10, 6.47, and 4.70) for T1, T2, T3, and T4, respectively. These results differed from the results of ²⁹, as the feed conversion rates of grass carp for the six treatments (25, 27, 29, 31, 33, and 35% crude protein) ranged between 3.30-3.57. ²⁸ recorded a 3.1 feed conversion rate for grass carp. Some results of the current study also differed from those of ¹⁸, where the best food conversion rate (1.73) was achieved at 40% protein in their laboratory study on grass carp. The results of the current study differed from those of ⁹, where the feed conversion rate for juvenile grass carp fed on a diet made with 26.37% protein was 1.3, which may be due to the difference in the initial weight.

CONCLUSIONS

Since the cost of fish production is mostly due to feed costs, and the highest component of the cost is protein, especially animal protein, the current experiment aims to find the lowest percentage of protein in the diets provided to grass carp that achieve best results in growth and productivity, and then encourage Iraqi culturists to cultivate this carp.

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