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Effect of the Feeding on Artificial Diet or Alfalfa Plant on the Growth of Grass Carp *Ctenopharyngodon idella* (Val. 1844) Fingerlings Cultivated in the Earthen Ponds

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Abstract: The current study aims to compare the growth of young grass carp *Ctenopharyngodon idella* fed on either artificial diet or alfalfa. The experiment was carried out in the earthen ponds of the Aquaculture Unit in the Agricultural Researches and Experiments Station (College of Agriculture, University of Basrah), which is located in the Al-Hartha district, approximately 16 km north of Basra (30^0 65' 64.6"N, 47^0 74' 79.5"E). The experiment begun from 26 July 2019 to 10 December 2019. Six ponds with an area of 600 square meters each were used for treatments. Fish were distributed equally to the culture ponds (600 fish per pond) at average individual weight of 6.3±0.28 g. Fish in artificial diet treatment were fed 5 % of the body weight and in alfalfa treatment 50 % of the body weight. Samples of fish were weighed every twenty days for the purpose of controlling growth parameters subsequently adjusting the daily feed provided based on the new weight. The growth criteria (final weight, weight gain, daily growth rate, specific growth rate, relative growth rate, feed conversion ratio and protein efficiency ratio) were calculated. Results of the current study showed that the water temperature is the most variable factor, for example, the highest temperature was 27.2ºC in July, while the lowest temperature was 18.7ºC in December. Results of growth criteria of the current study showed that grass carp in earthen ponds preferred alfalfa rather than the artificial diet, as the final average weight were 97.35, 47.18 g, the weight gain rate were 91.73, 40.75 g $fish^{-1}$, the daily growth rate were 1.27, 0.42 g day^{-1}, relative growth rate 1707.77, 629.99 %, specific growth rate were 3.92, 2.04 % day^{-1}, feed conversion ratio were 11.75, 2.51and protein efficiency ratio were 1.75, 0.45 for fish fed on alfalfa plant and the artificial diet respectively.
Key words: SGR, Feeds, Earthen ponds, Grass carp.

Introduction

Grass carp *Ctenopharyngodon idella* is a very popular species in freshwater aquaculture (Xie et al., 2018). Grass carp became the first fish for aquaculture in the world, which has the ability to withstand extremist environmental conditions, as well as the ease of cultivation, availability of nutritional requirements and its acceptance by the consumer, this led to the

success of its cultivation on a large scale, the global production of grass carp reached 10500 tons in 2018 (FAO, 2020).

Based on molecular studies Tan & Armbruster (2018) classified grass carp in the family of Xenocyprididae instead of Cyprinidae as it is now accepted (Froese & Pauly, 2021). Grass carp in natural environment, feed almost entirely on floating and/or submerged macrophytes and detritus, but in aquaculture, many aquatic, terrestrial plants and types of processed feeds were used (Zhao *et al.*, 2020). The young grass carp feed on crustaceans and small fish, but the adults can feed other sources in case of scarcity in vegetation, such as small fish, but in ponds, they prefer feed on plants, however they did not show an increase in weight when they were fed on diets of animal sources (Van Zon *et al.*, 1978; Chilton & Muoneke, 1992). Shireman et al. (1978). When different plants feeds compared with manufactured food, it was found that the alfalfa tend to increase the growth rates of grass carp and had significant difference from other treatments Nekoubin & Sudagar (2012). Zolfinejad *et al.* (2017) recorded the higher values of fish weight gain rate in treatments of duckweed and alfalfa compared to other aquatic and terrestrial plants. Feeding grass carp on plants results in healthier fish, reduces production cost, increases net profit, and is more environment-friendly ecosystem (Toutou *et al*, 2018). Salih *et al.* (2008) cultivated grass carp in earthen ponds and used alfalfa in their feeding. Mgbenka & Lovell (1986) fed grass carp larvae on a diet containing alfalfa powder at a rate of 19 %, they found that fish fed the diet containing alfalfa had high weight gain and lower feed conversion. Nekoubin & Sudagar (2012) studied the effect of manufactured and plant diets on the growth performance and survival rate of grass carp juveniles using different types of feeds including alfalfa, their results showed that the best performance recorded in fish fed on artificial diets compared to vegetable diets .

Taher (2017) conducted several laboratory experiments on feeding of grass carp, he found that the best growth of these fish is achieved when they fed with manufactured diets compared with aquatic plants alone or aquatic plants and diets. Abdullah *et al.* (2020) exhibited the preference of grass carp for three types of aquatic plants, namely *Azolla* plant, duckweed and Brahmi herb *Bacopa monnieri* at different temperatures, concluding that there are no significant differences in the preference of *Azolla* and duckweed plants, while the fish did not eat Brahmi plant. Al-Dubakel *et al.* (2020) recorded the highest weight achieved in grass carp when adding 5 % of *Azolla* plant powder, while the lowest weight was gained when adding 15 .%

Taher (2020a) found that the best growth rate with lowest feed conversion ratio were achieved when grass carp were farmed at a density of 0.41 fish m^{-2}, while the lowest growth rate with the highest food conversion ratio were at density 1.25 fish m^{-2}. Al-Dubakel *et al.* (2020) study the effect of partial replacement of fish meal with *Azolla* meal in grass carp feed. The current study aims to compare the growth performance of young grass carp fed with artificial diet or alfalfa in earthen ponds.

Materials & Methods

Study site

The experiment was conducted in the earthen ponds belonging to Aquaculture Unit at Agricultural Researches and Experiments Station (College of Agriculture, University of Basra), which located in the district of the Al-Hartha, approximately 16 km north of Basra (30^0 65' 64.6"N, 47^0 74' 79.5"E). This station includes four large ponds (2500 m^2 and 14 small ponds (600 m^2).

Environmental measurements

The temperature, pH, salinity and dissolved oxygen were measured using a digital device of the type Lovibond Senso Direct 150.

Experimental ponds

The water area of pond was 600 m^2 with depth of 1.5 meters each. Before the experiment started, all ponds were drained entirely for 10 days till complete dryness. Ponds were supplied with fresh water from one of the tributaries of the Shatt Al-Arab river. Water was supplied by electric pumps without supplemental aeration. Six ponds were used, the ponds were filled with water and left for several days for the purpose of developing the natural food. The water inlet and outlet pipes are provided with sieves to prevent the entering of wild fish or escaping of the reared fish.

Experimental fish

Young grass carp, with average weight of 6.3±0.28 g were transferred from Thi-Qar Governorate during 7 July 2019 to the Marine Science Center ponds in Basrah, and after seven days they were transferred to the Al-Hartha culture ponds. Fish were distributed in the ponds at a density of 1 fish m^{-2}. Three replications for artificial diet and the alfalfa plant were used. During 26 July 2019, a random sample (about 100 fish) was taken from each pond using a seine net and then fish were transported outside the pond site and transferred to a tank supplied with experimental water then they were returned to the ponds after taking the measurements of their weights.

Feeding management

The total weight of fish were measured in the ponds to calculate the daily food quantity for each pond, daily food quantity for ponds was calculated, as the fish in ponds 1, 2, and 3 were fed on artificial diet which manufactured by the feed production plant in the Agricultural Advisory Office of the College of Agriculture, University of Basrah. Table (1) shows the components, and the calculated chemical composition (g. kg^{-1}) on the basis of the dry weight of the diet used after adopting a feeding rate of 5 % body $weight^{-1}$, while the fish in ponds 4, 5 and 6 were fed with the alfalfa plant, which was obtained from the local markets. After adoption of a feeding rate at 50 which was obtained from the local markets. After adoption of a feeding rate at 50 %, due to high moisture content of alfalfa (95.55±0.78) and according to field observation, it was found that this feeding rate may be quit sufficient for fish, also because the small size of fish at the beginning of the experiment, unable them to consume all amount of alfalfa, the plants were cut using an electric cutting machine designed for this purpose until the fish were able to eat all parts of the alfalfa.

The daily feed quantity for the ponds was divided into three equal meals, the first feeding was in the morning, the second was during the midday and the third was at afternoon. The

ration was distributed manually throughout the experiment period. The fish measurement process was repeated every 20 days, as the daily feed quantity given to fish in all ponds was adjusted on the basis of the new weight of the fish.

Table (1): Ingredients and proximate composition of the artificial diets.

Ingredients	%
Fish meal	20
Wheat flour	35
Wheat bran	23
Soybean meal	20
Vit. & mineral premix	2
Proximate composition (% DM)	
Moisture	9.67±0.58
Crud protein	27.12±0.82
Crud lipid	6.11±2.47
Ash	7.61±0.54
Carbohydrates	46.82±2.35
Gross energy (Kcal.kg^{-1})	4272.50±60.10

Growth performance

At the end of the experiment during 10 December 2019, growth parameters were taken during the experimental period and the following equations were used to describe the growth performance of grass carp:

Weight gain:

$$WG = W2\ (g.fish^{-1}) - W1\ (g.fish^{-1})$$

Relative Growth Rate:

$$RGR = \frac{(W_2\ g\ .fish^{-1} - W_1\ g\ .fish^{-1})}{W1} \times 100$$

Specific Growth Rate:

$$SGR = \frac{(\ln W_2\ g\ \ fish^{-1} - \ln W_1\ g.fish^{-1})}{t_2 - t_1} \times 100$$

Where $\ln W_2$ is the natural logarithm of the final weight at the time T2, $\ln W_1$ is the natural logarithm of the initial weight at the time T_1 and T_2-T_1 is the period between the two weights.

Feed Conversion Ratio:

$$FCR = \frac{R\ g}{WG\ g}$$

Where R: weight of dry feed intake. WG: wet weight gain (live weight of fish).

PER

$$PER = \frac{WG\ g}{PI\ g}$$

Where WG: wet weight gain (live weight of fish).

PI: weight of protein intake.

Survival rate:

$$\text{Survival rate} = \frac{No.\ of\ fish\ alive}{Total\ No.\ of\ fish\ stocked} \times 100$$

Chemical analysis

The artificial diets and alfalfa were analyzed according to A.O.A.C. (2016). The moisture content was estimated by drying the samples at a temperature of 105 ^{0}C.

The proteins were estimated using the Microkjeldahl device. And the percentage of lipids was estimated using a Soxhlet apparatus in the presence of hexane as an organic solvent. The ash was estimated by burning the samples in Muffle furnace at 550 °C for 4 hours. Total carbohydrates were estimated according to the equation mentioned by Wee & Shu (1989):

$$\%COH = \%DM - (\%EE + \%CP + \%ASH)$$

Nitrogen free extract was calculated according to New (1987) as follow:

%NFE = %DM – (% EE + %CP + %ASH + %CF)

Where:

NFE = Nitrogen free extract

DM = Dry matter

EE = Ether extract or crude lipid

CP = Crude protein

CF = Crude fiber

COH = Total carbohydrate

Statistical analysis

All parameters were tested by using completely randomized design (CRD) and one-way analysis of variance (ANOVA). Significant results ($P \leq 0.05$) were tested using LSD to test significant difference between means. The statistical analysis was calculated by computer software SPSS package Version 22.

Results

The values of environmental parameters during the experimental period presented in table (2), as the water temperature was the most variable factor, the highest temperature was 27.2 °C recorded during July and the lowest temperature was 18.7 °C during December, pH value was 7.5 in December and was 8.4 in November, the water salinity ranged from 4.8 psu in September to 9.3 psu in July, and dissolved oxygen was in highest concentration (7.5 mg. $L^{-1)}$ in December, while the lowest concentration was 6.4 mg. L^{-1} in August.

The proximate composition analysis of the artificial diet and alfalfa are shown in table (3), there was a major difference between the two feeds in moisture, dry matter and fiber content, but protein content, lipid and ash were higher in the artificial diet compared to alfalfa which shows higher content of fiber, NFE and total energy content of both feeds were more or less similar.

Table (2): The environmental parameters during experimental period.

Date	Temp. (°C)	pH	Salinity (psu)	DO (mg. L^{-1})
27Jul.	27.2	8.1	9.3	6.7
15Aug.	25.9	7.9	6.3	6.4
3Sep.	26.1	8.1	4.8	6.8
25Sep.	26.6	7.8	5.1	6.7
17Oct.	23.6	8.1	5.6	7.1
9Nov.	19.6	8.4	5.6	6.9
10Dec.	18.7	7.5	5.1	7.5

Table (3): Proximate composition of artificial diet and alfalfa (Mean± SD).

Proximate composition (%)	Alfalfa	Artificial diet
Moisture	95.55 ±0.78	9.30 ±0.42
Dry matter	4.45 ±0.78	90.70 ±0.42
Crud protein	22.37 ±0.54	26.49 ±0.58
Crud lipid	2.10 ±1.35	6.29 ±2.84
Ash	5.16 ±0.93	7.55 ±0.64
Crud fiber	21.48 ±11.62	4.45 ±0.21
NFE	46.42 ±9.50	45.35 ±0.92
Gross energy(Kcal. kg^{-1})	3275.7 ±288.15	3837.05 ±187.17

Final weight rates, weight gain rate, daily growth rate, specific growth rate, relative growth rate, feed conversion ratio and protein efficiency ratio during the experiment period presented in table (4). Results clearly exhibited that grass carp fed on alfalfa had positive effects on the growth parameters and showed

significant difference (P≤0.05) compared with artificial diet. In both feeds, it was not observed that the fish rejected to ingest the experimental feeds.

Table (4): Growth performances and nutrient utilization of grass carp fed on artificial diet and Alfalfa (Mean± SD).

Parameters	Artificial diet	Alfalfa
IW (g)	6.43 [a] ±0.55	5.62 [b] ±0.85
FW (g)	47.18 [a] ±9.27	97.35 [b] ±21.6
WG (g)	40.75 [a] ±8.74	91.73 [b] ±22.42
DGR (g day^{-1})	0.42 [a] ±0.09	1.27 [b] ±0.31
RGR (%)	629.99 [a] ±90.21	1707.77 [b] ±629.93
SGR (% day^{-1})	2.04 [a] ±0.13	3.92 [b] ±0.56
FCR	2.51 [a] ±1.21	11.75 [b] ±4.43
PER	1.75 [a] ±0.74	0.45 [b] ±0.16
Survival rate (%)	100 [a]	100 [a]

Data in each row with different letters are significantly different (P≤0.05).

Results showed that the highest final weight rate was recorded in alfalfa treatment (97.35±21.6 g), while the lowest final weight average was in artificial diet (47.18±9.27 g) and the weight gain was the highest (91.73 ±22.42 g) in alfalfa feed, while the lowest weight gain was recorded in artificial diet (40.75±8.74 g). The daily growth rate was 0.42 ±0.09 and 1.27 ±0.31 g.day^{-1} in the artificial diet and alfalfa feed, respectively, and the relative growth rate was 629.99±90.21 % in the artificial diet and 1707.77±629.93 % in alfalfa. The results of the current study also showed that the highest specific growth rate was in alfalfa 3.92±0.56 % day$^{-1,}$ while the lowest specific growth rate was in the artificial diet (2.04±0.13% day^{-1}). Feed conversion ratio of the grass carp fed on the artificial diet was 2.51±1.21 which was superior than alfalfa, the former recorded a feed conversion ratio of 11.75±4.43 at the level of food basis, protein efficiency ratio was 1.75±0.74 and 0.45±0.16 in the artificial diet and alfalfa respectively.

The results of the current study showed that there was fluctuations in water temperature and salinity, which greatly have effects on the growth at a constant culture density, and this is consistent to what was found by Saleh *et al.* (2008) who showed the growth fluctuations according to the change in temperature, as they recorded a turbulence in the growth of grass carp was observed from mid-June (7.6±0.312 g day^{-1}) and started to decrease in January 1.5±0.221 g day^{-1} at temperature range 17-27°C. Some researchers confirmed that the optimum temperature for the highest level of feeding in grass carp was 21-26 °C (Colle *et al.*, 1978; Shireman & Mciena, 1981), these results matched our findings in current study. The highest growth rate of grass carp was at temperature range (24-29°C), while the growth rate decreased at lower temperatures, this results correspond to study of Hemlata *et al.* (2016) who recorded a temperature rate of 24.4^{o}C, also with study of Taher (2020b), who found that the best rate of feed intake for grass carp was at a temperature of 25^{o}C, the range of temperature in the present study, except the last two months were within the suitable range for grass carp growth. With respect of salinity, the current study was dissimilar from Hossain *et al.*

(2020) who cited that the salinity of the pond water (1 psu) that was much lower than what was recorded in the current study, and the pH value was lower compared to the current study (7-7.1). The dissolved oxygen concentration in the current study was higher in comparison with previous studies, this may due to the water temperature that was lower. It is known that the lower the water temperature, the higher its ability to keep oxygen molecules. The highest concentration of salinity in the current study was recorded during July which reached 9.3 psu and the lowest concentration (4.8 psu) was at the beginning of September. Kilambi & Zdinak (1980) found that grass carp fed well and active at the at the acclimation salinities between 3 to 9 psu, also Al-Seyab (1996) showed that grass carp has the ability to withstand changes in salinity up to 12 psu without show signs of stress, while Taher (2020a) recorded rates of salinity concentration lower than what was recorded in the current study.

Results of the current study showed that alfalfa was superior than artificial diet in respect to grass carp growth parameters, this could be related to the small size of fish. Under suitable conditions, grass carp will eat more than its individual weight of plant material on a daily basis and the daily ration (total mass feed consumed per day/fish mass) may reach 49.9% (Toutou *et al.*, 2018; Nekoubin & Sudgar, 2012), this finding is consistent with the 50% feeding rate used in the present study. Zhao *et al.* (2020) stated that chopped leaves and stems of several plants proved to be potential food for this species, this finding differed from the study of Nekoubin & Sudgar (2012) in data of the final weight rate, weight gain, daily growth rate and specific growth rate, as the researchers indicated that the artificial diet was the best in all these criteria, as well as with other studies such as Van Zon *et al.* (1978), Chilton & Muoneke (1992) and Taher (2017), who indicated that grass carp prefer artificial diet rather than plant feeds. Results of the current study differed from study of Sutton (1981) in daily growth rate that reached 0.58 g/day in grass carp fed on alfalfa in the closed system, in the current study qualitative growth rate was closer to what was recorded in the study of Devaraj *et al.* (1986) when grass carp was fed on alfalfa, while it was superior in qualitative growth rate when grass carp was fed on *Moringa oleifera* L. in same study. The current study recorded a lower daily growth rate than what was recorded in the study of Majhi *et al.* (2006) when grass carp was fed on Azolla plant, which reached to 1.65 g/ day. This is due to the high protein content of Azolla plant and its softness. The current study is consistent to the study of Filizadeh *et al.* (2004) stated that grass carp prefer feeding on plants, as small fish select softer plant tissue while bigger fish eat a wide variety of tough and fibrous plants, the current study confirmed what it was found by Nekoubin & Sudgar (2013), who cited the superiority of grass carp fish fed on the alfalfa in all growth parameters on those fed on artificial diets. In addition, experiments in current study performed better daily growth rate in grass carp fed on alfalfa, it reached 0.33 g. day^{-1}.

As for the value of the feed conversion ratio, the current study has similar result to the study of Nekoubin & Sudgar (2013), they showed an increase in the value of the feed conversion ratio of alfalfa compared to the manufactured diet. Taher (2017) noted that feeding the young grass carp on the plant only gave a negative daily growth rate of -0.22 $g.day^{-1}$, while the

current study recorded a highly daily growth rate when fed on alfalfa that is higher than that fed on a manufactured diet. Saleh *et al.* (2008) recorded higher relative growth rate of 7591.6 %.day^{-1} in grass carp fed on alfalfa in earthen ponds compared to recent study. The current study showed that the percentage of protein efficiency of the artificial diet is higher than that of the alfalfa, tending to a decrease in weight gain with an increase in the amount of protein provided to fish, as the amount of total protein provided to fish in the alfalfa was much greater than the total protein provided in the artificial diet. The present values of the protein efficiency ratio for the artificial diet are closer to what was reported in the study of Yang *et al.* (2013) who recorded 1.65-2.07 for diets with different percentages of phosphorus in feeding grass carp, as well as Javed &Watanabe (2000) who recorded a protein efficiency ratio of 1.41-2.08 using diets with different protein content (23.16-33.15 %) in grass carp.

The results of the current study differed from the study Du *et al.* (2006), who tested the effect of different feeding ratios on growth, nutrition efficiency and body composition using a diet with a protein content of 35.8 %, as the feeding percentage 2.5 % recorded protein efficiency ratio of 0.48 and this difference is due to a difference in the percentage of protein. The protein used in Du *et al.* (2006) experiment was combined with the percentage of protein used in the current study. The current study of grass carp fed on an artificial diet at a feeding ratio of 5 % recorded the efficiency of a protein, same result was recorded by Halbus (2018). The current study recorded a lower protein efficiency ratio for the alfalfa in comparison of Köprücü & Sertel (2012), whom they used different plants with a protein content of 40 %, and protein efficiency ratios were 1.32-2.08. Al-Shkakrchy & Ahemed (2013) recorded a protein efficiency ratio (0.77) higher than what was recorded in the current study, in their experiment, they used duckweed in feeding of grass carp in laboratory, and it perhaps due to the high protein content (29.69%) in the duckweed when compared with the alfalfa and its tenderness, which enables young grass carp to eat all parts of the plant, furthermore it contains essential amino acids, which are similar to the amino acids in chicken eggs.

Conclusion

Grass carp fed alfalfa feeds showed the best growth performance and nutrient utilization (expect FCR because of its high moisture content) and could be advised to use it as commercial feeds in earthen ponds for young size grass carp, while the artificial diets could be more useful for the large size of grass carp.

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Conflict of interest

The authors declared that they have no conflict of interest.

Ethical approval

All applicable national and international guidelines for the care and use of animals were followed.

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تأثير الغذاء الاصطناعي ونبات الجت على نمو اصبعيات الكارب العشبي *Ctenopharyngodon idella* (Val. 1844) في الأحواض الترابية

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المستخلص : تهدف الدراسة الحالية إلى مقارنة نمو صغار الكارب العشبي *Ctenopharyngodon idella* المغذى على الغذاء الاصطناعي ونبات الجت. أجريت التجربة في الاحواض الارضية التابعة لوحدة الاستزراع المائي في محطة البحوث والتجارب الزراعية (كلية الزراعة- جامعة البصرة) الواقعة في منطقة الهارثة على بعد حوالي 16 كم شمال البصرة (65 '64.6 ' 30^0 شمالا، '79.5' 74 47^0 شرقا) للفترة من 26 تموز 2019 إلى 10 كانون الاول 2019. تم استخدام ستة أحواض بمساحة 600 متر مربع لكل واحد . تم توزيع الأسماك بالتساوي على أحواض الاستزراع (600 سمكة لكل حوض) بمتوسط وزن 6.3 ± 0.28 غم. تم تغذية الأسماك في الأحواض 1 و 2 و 3 على غذاء اصطناعي بمعدل تغذية 5٪ من وزن الجسم. تم استخدام نبات الجت من السوق المحلية لتغذية أسماك الأحواض 4 و 5 و 6 بنسبة تغذية تصل إلى 50٪ من وزن الأسماك. تم وزن عينة من الأسماك كل عشرين يوما لغرض مراقبة معاملات النمو وتعديل الغذاء اليومي من العلف بناء على الوزن الجديد. تم حساب معايير النمو (الوزن النهائي، الزيادة الوزنية، معدل النمو اليومي، معدل النمو النوعي، معدل النمو النسبي، معدل التحويل الغذائي ونسبة كفاءة البروتين). أظهرت نتائج الدراسة الحالية أن درجة حرارة الماء هي العامل الأكثر تبايئا ، وكانت أعلى درجة حرارة 27.2˚ م في تموز، وأدنى درجة حرارة كانت 18.7 ˚ م في كانون الاول. أظهرت نتائج معايير النمو للدراسة الحالية أن أسماك الكارب العشبي في الأحواض الأرضية فضلت نبات الجت على العليقة الاصطناعية، حيث كان متوسط الوزن النهائي 47.18، 97.35غم ، معدل زيادة الوزن 40.75، 91.73 غم سمكة $^{-1}$، كان معدل النمو اليومي 1.27 ، 0.42 غرام. يوم $^{-1}$، معدل النمو النسبي 1707.80 ، 629.99 %، معدل النمو النوعي 2.04 و 3.92 % يوم$^{-1}$، نسبة التحويل الغذائي 2.51 و 11.75 ونسبة كفاءة البروتين 0.45 و 1.75 لكل من الأسماك المغذاه على نبات الجت والغذاء الاصطناعي على التوالي.

الكلمات المفتاحية: SGR، الغذاء، الاحواض الارضية، الكارب العشبي.