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Effect of different dietary protein levels in artificial diets on the growth juvenile common carp (*Cyprinus carpio* L. 1758) in laboratory

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Abstract

The experiment was conducted to examine the impact of various protein levels on the growth performance of juvenile common carp Cyprinus carpio. Diet formulated in the laboratory to provide crude protein levels of 30% (T1), 35% (T2), 40% (T3) and 45% (T4). The juveniles of common carp (Initial average weight 1.12±0.11 g). Twelve plastic aquaria (Three aquaria for each treatment) of 100 liters capacity were used and stocked with 15 fish each. The aquaria were equipped with electric air pumps. A feeding ratio of 5% of fish weight was used to feed the fish six days a week during the 60-day experiment. To adjust the feeding rate according to the new mean body weight, the weight of every fish in each replicate was measured every 15 days. Results of the current experiment revealed final average weights of 1.61, 1.82, 2.01 and 2.00g for T1, T2, T3 and T4 respectively. The highest weight gain was recorded by fish in T3 was 0.89 g, while the lowest (0.52 g) was reached by fish of T1. The highest daily growth rate (0.015 g /day) was reached by fish of T3 while the lowest (0.010g/day) was reached by fish of T1. Specific growth rates were 0.649 % /day for T1, 0.851 % /day for T2, 0.974 % /day for T3 and 0.907 % /day for T4. Fish from T3 had the best feed conversion rate (4.26), whereas fish from T1 showed the poorest feed conversion rate (5.91). In conclusion the best growth results were achieved by fish of T3 compared with other treatments especially T1.

Keywords: Protein Levels, FCR, Diet, Growth Performance, Juvenile, Common carp.

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Introduction

Improving water quality of in fish culture, it is necessary to improve nutritionally balanced and affordable feed (Abdel-Tawwab, 2012). Al- Zahrani *et al*. (2013) mentioned that the type and quantity of ingested feed on affected growth rate, feed conversion rate and proximate body composition. It is well known that fish using protein as an energy source, and it is necessary for promoting fish growth (Luo *et al*., 2004). Fish`s production can be increased by using sufficient protein levels in the diet, however higher protein levels will be converted to energy and release more nitrogen (Luo *et al*., 2004). Yun *et al*. (2015) noted that the understanding of protein needed for cultivated fish is crucial for well-balanced and affordable feeds. The majority of information regarding the nutritional needs of fish is based on short-term studies and it begins with small-sized fish (Giri *et al*., 2003). Yun *et al*. (2015) pointed out that one of the most important elements influencing fish growth in intensive aquaculture is feed quality.

Since protein is the most crucial and costly component of a diet, most studies pay more emphasis on protein dietary requirements. Additionally, the feeds of Juveniles contain a higher level of protein compared with large individuals because energy demands are higher in the early stages of life. Gandotra *et al*. (2018) mentioned that knowledge of level feed protein need decreased according to fish age, the appropriate protein amount for economically viable and healthy juvenile productionWilson (2003) recorded that fish need feeds with high protein content compared with other farm animals, whenever German (2011) determined the protein requirements of fishes as 2-4 times more than other vertebrates. Islam *et al*. (2004) referred that the fish development rate at various life stages, particularly larval stages, is significantly influenced by protein, which is the primary source of energy. It had been pointed out from different studies that the dietary protein requirements of fish ranged between 24%-70% depending on species and age (Teles *et al*., 2019). Steinberg (2022) stated that in spite of protein affected growth, health and feed consumption of water organisms, the excess protein may decrease growth, reduce immunity and deteriorate water quality.

FAO (2018) pointed out that the common carp, *C. carpio* is one of the main aquaculture species in many European, Latin American and Asian countries. It is one of the most cultivated species due to its quick growth rate, good environmental tolerance, tolerance of handling and higher fish densities and also the capacity to use artificial diet effectively (Mohapatra & Patra, 2014). Vilizzi *et al*. (2015) mentioned the introduction of common carp to different ecosystems such as lakes, dam lakes, rivers and streams, while Ljubojević *et al*. (2016) referred that this species was the most important fish in the ecosystems of Eastern Europe. Khan *et al*. (2016) mentioned that this species was introduced to many countries including Europe, Australia and North America.

The production of cultivated common carp around the world in 2020 was 4236.3 thousand tons and it was the fourth cultivated species after grass carp, *Ctenopharyngodon idella*, silver carp, *Hypophthalmichthys molitrix* and Nile tilapia, *Oreochromis niloticus*

(FAO, 2022). Till now, Iraq's fish production don't agree within world production and the production of common carp is far less than in other nations. According to recent information, common carp consist now make up more than 95% of cultivated fish species in Basrah province, while Jassim *et al.* (2013) mentioned that it is consisted about 63.5%. The aim of the current study was to determine the standard protein level in the diet of common carp that leads to better growth.

Materials And Methods

The varying effect of dietary protein levels on the growth of juvenile common carp was studied in vitro using an artificial diet. The diets were manufactured by the feed plant of the Agricultural Consultant Office belonging to Agriculture College with different levels of crude protein to provide crude protein ratios of 30%, 35%, 40% and 45% (Table, 1). The chemical analysis of the different diets was carried out in the feeding Laboratory, located in the Marine center of Basrah University, and three replicates were taken from each of the four treatments. Common carp juveniles (initial average weight 1.12±0.11 g). Twelve plastic aquaria (Three aquaria for each treatment) of 100 liters capacity were used and stocked with 15 fish each.

The aquaria were equipped with electric air pumps. Fish were acclimated to the experimental apparatus and its parameters for eight days prior to the experiment's commencement. Weighing fish was done with a ZH 8257 electronic balance. The experimental diets included, T1 (30% protein), T2 (35% protein), T3 (40% protein) and T4 (45% protein) were used in feeding trail of current experiment. During the 60-day experiment, fish were fed six days a week at a feeding ratio of 5% of their body weight. To adjust the food to the new mean body weight, the weight of every fish in each replicate was measured every 15 days. During the experiment period that, five sampling data were used to calculate using the following equations (Jobling, 1993):

$$Weight\ gain\ (WI, g) = FW - IW$$

$$Daily\ growth\ rate\ (DGR, g/day) = (FW - IW) / days$$

$$Specific\ growth\ rate\ (SGR, \%/day) = 100 \times (ln\ FW - ln\ IW) / days$$

$$Feed\ conversion\ rate = Food\ intake / weight\ increment$$

Where: FW = Final fish weight (g); IW = Initial fish weight (g)

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This experiment's results were obtained using a completely randomized design. Analysis of variance (ANOVA) was used to test for mean differences, and SPSS program Ver. 26 was used to test for significant differences using the LSD test at the 0.05 probability level.

Table 1: Experimental ingredients and protein levels diets.

Treatments	Feed ingredients (%)					Protein percentage%
	Fish meal	Wheat flour	Corn flour	Soybean meal	Premix (vitamins and minerals)	
T1	35	25	25	13	2	30
T2	41	25	12	20	2	35
T3	45	15	6	32	2	40
T4	50	2	2	44	2	45

Results

Environmental factors rates were measured, including, temperature where it was recorded $8.12_{\pm 0.31}$ mg /l, salinity was $2.38_{\pm 0.53}$ PSU and pH was $8.19_{\pm 0.24}$. Table (2) illustrated the average fish weight for 12 aquaria during the experiment. Initial average weights of the four treatments were (1.09, 1.09, 1.12, 1.16) g respectively.

Table (3) shows the growth criteria of different aquaria and their average for the four treatments. Final average weights were (1.61, 1.82, 2.01 and 2.00) g for T1, T2, T3 and T4, respectively (Fig.1). Results for final weights showed statistically significant differences ($P \leq 0.05$) between T1 and the other three treatments, while there were no significant differences ($P > 0.05$) between T2, T3 and T4. The highest weight increment reached by fish in T3 was 0.89 g, while the lowest 0.52 g was reached by fish of T1. A statistical examination of the weight increase results shows that T1 and the other three treatments differed significantly ($P \leq 0.05$), whereas, T2, T3, and T4 did not differ significantly ($P > 0.05$).

The highest daily growth rate (0.015 g /day) was reached by fish of T3 while the lowest (0.010 g /day) was reached by fish of T1 (Fig. 2). The results of a statistical examination of daily growth rates appear that T1 and T2 differed significantly from T3 and T4 ($P \leq 0.05$), while there were no significant differences ($P > 0.05$) between T1 and T2 and also between T3 and T4. Specific growth rates were 0.649 % /day for T1, 0.851 % /day for T2, 0.974 % /day for T3 and 0.907 % /day for T4 (Fig. 3).

A statistical analysis of the results for a certain growth rate revealed that there were no significant differences ($P > 0.05$) between T1 and T2 or between T3 and T4, but there were significant disparities ($P \leq 0.05$) between T1 and T2 with T3 and T4. The best feed

conversion rate (4.26) was reached by fish of T3, while the worst feed conversion rate (5.91) was reached by fish of T1 (Fig. 4). A statistical analysis of the feed conversion rate data revealed no significant difference (P>0.05) between T2 and T3 or between T3 and T4, however there were significant differences (P≤0.05) between T1 and the three treatments and between T2 and T4.

Table 2: Fishes weight (g) during the experiment.

Date	Average Fish Weight (g)											
	T1A1	T1A2	T1A3	T2A4	T2A5	T2A6	T3A7	T3A8	T3A9	T4A10	T4A11	T4A12
23/5/2022	1.09 ±0.07	1.07 ±0.24	1.12 ±0.22	1.09 ±0.22	1.08 ±0.21	1.11 ±0.19	1.11 ±0.15	1.11 ±0.19	1.14 ±0.10	1.15 ±0.30	1.16 ±0.22	1.17 ±0.28
7/6	1.16 ±0.19	1.16 ±0.25	1.16 ±0.20	1.23 ±0.26	1.20 ±0.23	1.27 ±0.21	1.33 ±0.21	1.33 ±0.26	1.35 ±0.13	1.32 ±0.29	1.32 ±0.22	1.36 ±0.32
22/6	1.27 ±0.43	1.32 ±0.33	1.33 ±0.47	1.43 ±0.39	1.38 ±0.48	1.55 ±0.37	1.54 ±0.34	1.57 ±0.38	1.66 ±0.40	1.48 ±0.32	1.50 ±0.27	1.55 ±0.37
7/7	1.42 ±0.43	1.49 ±0.40	1.48 ±0.68	1.61 ±0.39	1.58 ±0.53	1.75 ±0.37	1.79 ±0.39	1.79 ±0.38	1.85 ±0.43	1.78 ±0.59	1.75 ±0.61	1.72 ±0.78
22/7	1.58 ±0.49	1.65 ±0.44	1.61 ±0.71	1.78 ±0.43	1.76 ±0.60	1.93 ±0.42	1.97 ±0.40	1.97 ±0.48	2.09 ±0.57	1.97 ±0.78	1.96 ±0.81	2.07 ±0.67

Table 3: Growth criteria of different treatments in the experiment.

Growth Criteria	T1 (P 30%)			T2 (P 35%)			T3 (P 40%)			T4 (P 45%)		
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
FW	1.58	1.65	1.61	1.78	1.76	1.93	1.97	1.97	2.09	1.97	1.96	2.07
Average	1.61a			1.82b			2.01b			2.0b		
WI (g)	0.49	0.58	0.49	0.69	0.68	0.82	0.86	0.86	0.95	0.82	0.80	0.90
Average	0.52a			0.73b			0.89b			0.84b		
DGR (g/day)	0.0082	0.0097	0.0082	0.0115	0.0113	0.0137	0.0143	0.0143	0.0158	0.0137	0.0133	0.0150

Average	0.010a			0.012a			0.015b			0.014b		
SGR (%/day)	0.6147	0.7208	0.6112	0.8174	0.8139	0.9219	0.9561	0.9561	1.0102	0.8971	0.8742	0.9509
Average	0.649a			0.851a			0.974b			0.907b		
FCR	6.35	5.67	5.72	4.99	4.49	4.35	4.35	4.45	4.09	4.51	4.68	4.18
Average	5.91a			4.81b			4.26bc			4.46c		

*Different letters in one row is significantly different (P≤0.05).

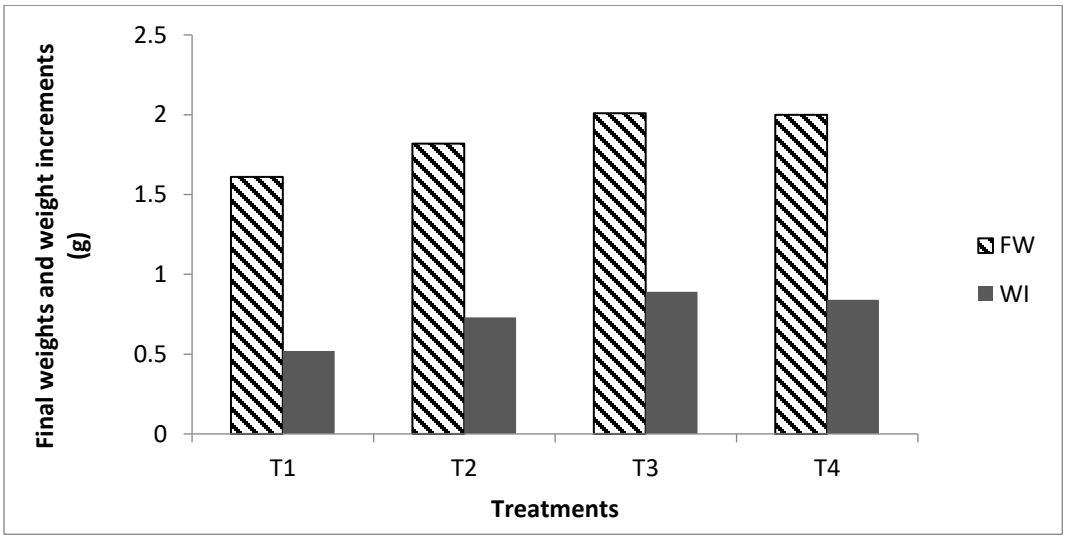

Figure 1: Final weights and weight gain of common carp juveniles at different treatments.

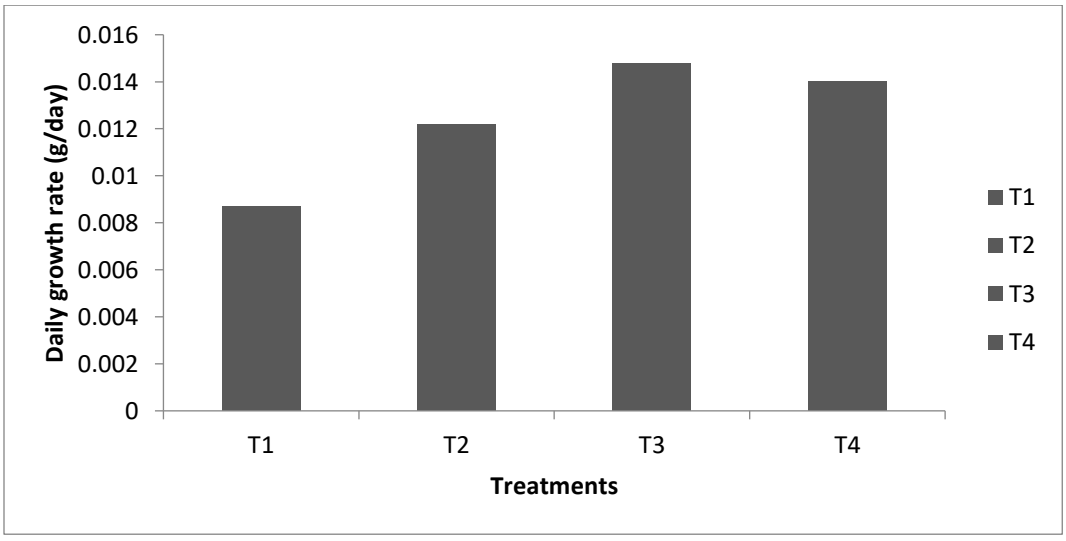

Figure 2: Daily growth rate of common carp juveniles at different treatments.

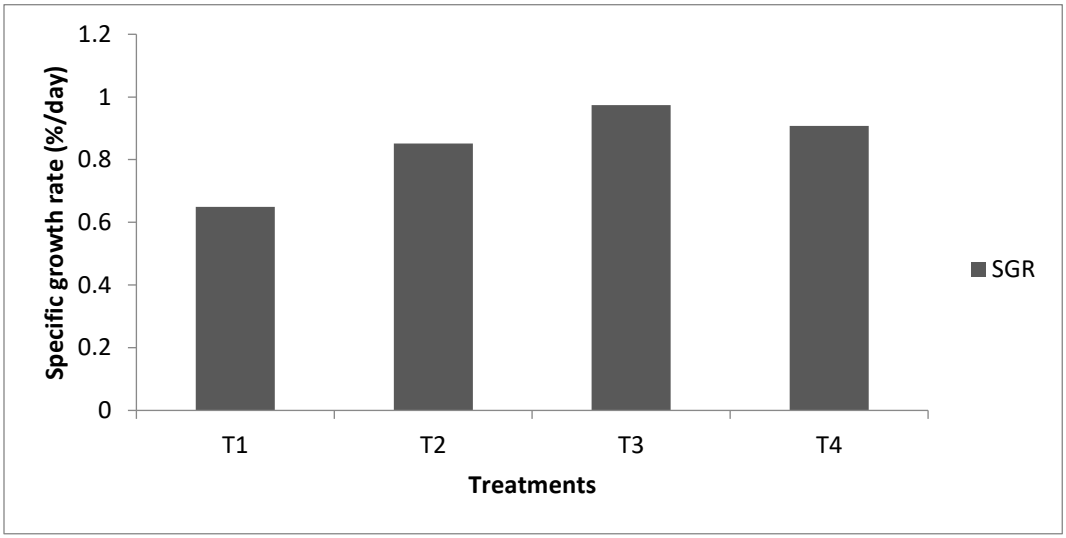

Figure 3: Specific growth rate of common carp juveniles at different treatments.

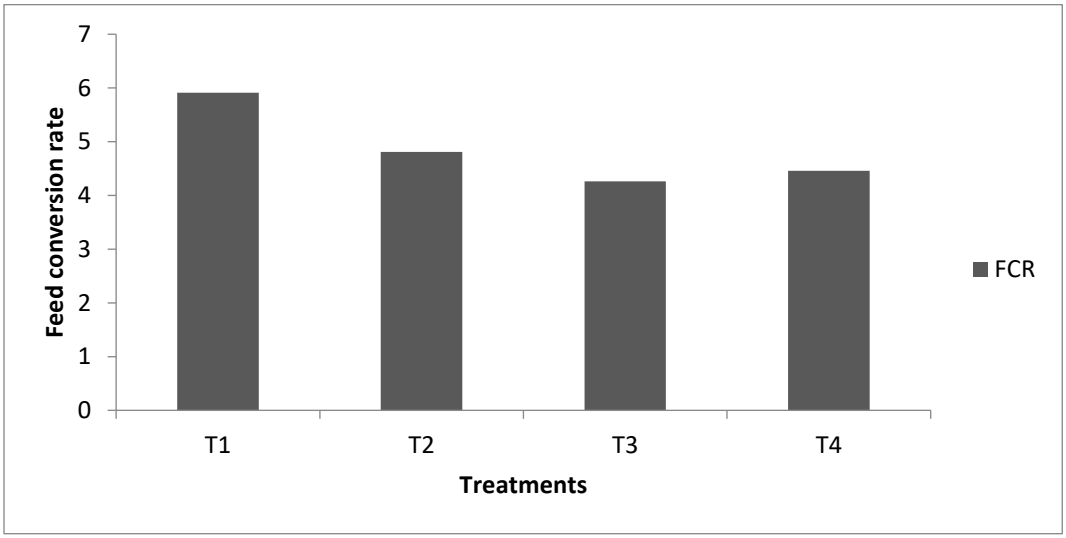

Figure 4: Feed conversion rate of common carp juveniles at different treatments.

Discussion

Mahfuj *et al.* (2012) stated that the first stages of fish rearing are a critical period for the successful culture of fish, especially with respect to the cultivated species of cyprinids. The current experiment's findings showed that juveniles given a diet with a protein level of 40% met the greatest growth criteria, followed by those fed a diet with a protein level of 45%. Juveniles fed a diet with a protein level of 30% met the lowest threshold. This means that the juvenile common carp need high protein ratio in their feeds compared with large fish. That may be related to the juveniles feed generally contains higher level of protein because the latter and energy demand are higher in the early stages of life. So, it is essential to recommend the suitable protein level of juvenile feed for economic production of

healthy juveniles and maximize its lifespan as well (Gandotra, *et al.*, 2018). Many researchers worked the common carp recommended protein ratio between 25-30%.

Taher (2023) found the same outcomes as the current experiment for common carp larvae, showing that the larvae fed a diet with a 40% protein level met the highest growth requirements, followed by those provided a diet with a 45% protein level. It is well known that excessive excretion of protein requires high energy, so higher protein level may be decreased the growth of fish. Protein requirements of fish depend upon environmental factors and feeding ratio and the moderate protein requirement was 42% (Steinberg, 2022).

The dietary protein requirements for major carp *Cirrhinus mrigala* fingerlings range from 30% protein in fishes cultivated in earthen ponds to over 45% in the case of small fishes (Inayat and Salim, 2005). Al-Akidi (2008) indicated that if protein ratios exceed the ideal level in common carp diets, the growth rate will be lower. It is commonly recognized that giving fish a lot of protein would cause the amine group to be removed from the protein, which is followed by an exchange of body energy that causes the fish's body weight to decrease. Mocanu *et al.* (2015) found that the growth performance of common carp was significantly improved at 48% and 35% protein level compared with 30%.

Maqbool (2017) stated that best growth performance achieved by mirror carp (*C. carpio*) fed a diet with 40% protein ratio comparing with (25, 30, 35, 45 and 50) %, and the optimum dietary protein ratio for common carp was 41.5%. Gandotra *et al.* (2018) pointed out that the best growth performance of common carp larvae was achieved by diets of protein level 40% and 45% comparing with 25%, 30% and 35%. Cordeli *et al.* (2019) mentioned out that weight gain and specific growth rate were increased significantly as dietary protein levels increased and the best result for common carp (initial weight 2.98 g) was at 50% protein comparing with other ratios. The best growth criteria achieved by common carp (initial weight 93.27 g) fed diets with 15% fish meal compared with 23% and 10% (AL-Bachry *et al.*, 2020).

Tamta *et al.* (2021) reported that, as compared to the control diet, the fish group fed a high-protein and lipid-incorporated feed achieved improved growth metrics for both common carp and Amur carp (*Cyprinus rubrofuscus*). Dabrowski (1977) stated that the optimum protein level in the feed of grass carp juveniles was 41-43%, while Ghazala *et al.* (2011) pointed out that from an economic view the protein level for young grass carp of 40% was better than 20, 30 and 35%. Assal *et al.* (2023) found that best growth performance of grass carp cultivated in earthen ponds reached by fish fed on diet with 35% protein level comparing with 20%, 25% and 30%.

Khattab *et al.* (2001) observed the growth performance that became better with increasing of protein level in the diet of Nile tilapia and found positive relationship between growth performance and protein levels. The results of the current experiment don't agree with the results of El-Saidy and Gaber (2005), where they didn't recorded any increase in the daily growth rate of Nile tilapia with increasing dietary protein levels.

Moreover, the findings of current experiment agree with It matches the results obtained of Nandesha *et al.* (2002) on common carp and the findings of Arun and Yakupitiyage (2003) on the Nile tilapia.

Abdel-Tawwab *et al.* (2010) were concurred with the same findings for Nile tilapia fed on protein rich diet that had better growth performance. Abdel-Tawwab (2012) pointed out that protein levels were positively affected the growth criteria of Nile tilapia and the best feed conversion rates were achieved with 45% and 35% protein ratio comparing of 25%. Elfeki *et al.* (2018) reported that the Nile tilapia (*O. niloticus*) fry, of both mono and mixed sex, grew best on a diet of 45% protein, then 50%, and the worst on a diet of 35% protein. The lack of a significant difference in growth between T3and T4 may be due to the dietary protein requirement of fish cannot be dissociated from the protein-to-energy balance (P/E) of the diet (Watts, et al., 2012). Since an optimal dietary P/E ratio will maximize the allocation of protein for growth and minimize its use as energy source for metabolic purposes (Fernandes, *et al.*, 2016).

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تأثير استعمال اغذية مختلفة نسب البروتين في نمو يافعات الكارب الشائع *Cyprinus carpio*

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المستخلص

اجريت التجربة الحالية لمعرفة تأثير مستويات مختلفة من البروتين في غذاء يافعات اسماك الكارب الشائع على اداء نموها، صنعت العليقة مختبريا للحصول على نسبة بروتين 30% في العاملة الاولى و35% في المعاملة الثانية و40% في المعاملة الثالثة و45% في المعاملة الرابعة. وضعت اليافعات بمعدل وزن (1.12 ±0.11) غم في 12 حوض بلاستيكي سعة 100 لتر (ثلاث مكررات لكل معاملة) بواقع 15 سمكة/ مكرر. زودت الاحواض بمضخات تهوية واستغرقت التجربة 60 يوم غذيت فيها الاسماك ستة ايام في الاسبوع بنسبة تغذية قدرها 5% من وزن الجسم. وزنت الاسماك كل 15 يوم لغرض تعديل كمية الغذاء اليومي وفقاً للأوزان الجديدة. اظهرت نتائج التجربة الحالية ان معدل وزن الاسماك النهائي التي بلغته اليافعات هو (1.61 و1.82 و2.01 و2.00) غم للمعاملات الاولى والثانية والثالثة والرابعة على التوالي. اعلى زيادة وزنية (0.89 غم) تم الحصول عليها من قبل اسماك المعاملة الثالثة واقل زيادة وزنية (0.52 غم) تم الحصول عليه من قبل اسماك المعاملة الاولى. حصل اسرع نمو يومي (0.015 غم/ يوم) في اسماك المعاملة الثالثة وابطأ نمو يومي (0.010 غم/ يوم) كان في المعاملة الاولى. بلغ معدل النمو النوعي 0.649 %/ يوم للمعاملة الاولى و0.851 %/يوم للمعاملة الثانية و0.974 %/يوم للمعاملة الثالثة و0.907 %/يوم للمعاملة الرابعة. بلغ افضل معدل تحول غذائي 4.26 في المعاملة الثالثة مقارنة بمعدل قدره5.91 في المعاملة الاولى. نستنتج من نتائج التجربة الحالية ان افضل اداء للنمو هو في اسماك المعاملة الثالثة مقارنة ببقية المعاملات وخصوصا المعاملة الاولى.

الكلمات المفتاحية: نسب بروتين، نسبة التحويل الغذائي، عليقة، اداءالنمو، يافعات، كارب شائع.