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The Effect of garlic meal as prebiotic on the growth and survival of young common carp *Cyprinus carpio* cultivated in earthen ponds

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Abstract - The current experiment was conducted in eight earthen ponds (600 m2), and each pond stocked with 1500 young common carp at average weight of 13.2 g. The experiment is conducted to investigate the differences in survival rate and growth performance for these young fishes fed diets with different ratio of dried garlic meal as additives [without additives (C), addition of 0.5% garlic (T1), addition of 1% garlic (T2), addition of 1.5% garlic (T3)]. Results of current experiment revealed that highest survival rate (97.8%) was achieved by fish in C and lowest 96.0% was achieved by fish in T3, while highest daily growth rate 0.3744 g/day was achieved by fish in T3 and lowest 0.3320 g/day was achieved by fish in T2. Feed conversions of all treatment were 2.74, 2.85, 2.82 and 2.38 for C, T1, T2 and T3 respectively. Statistical analysis of the results for survival rate, fe141ed conversion rate and all growth criteria studied in current experiment proved that there were no significant differences (P>0.05) between control and other three treatments and also between these treatments. The growth pattern for young common carp before the experiment was negative allometric were b value was 2.6496, while it was positive allometric for all treatments after experiment. There were no significant differences (P>0.05) in relative condition factor between before and after the experiment and also between all treatments.

تأثير الثوم كسابق حيوي في نمو وبقاء صغار الكارب الشائع Cyprinus carpio المستزرعة في الاحواض الارضية

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المستخلص - اجريت التجربة الحالية ثمانية احواض ارضية 600 متر مربع ووضع في كل حوض 1500 سمكة كارب شائع بمعدل وزن 13.2 غم. اجريت الدراسة الحالية لغرض اختبار تأثير اضافة مسحوق الثوم بمستويات مختلفة في نمو وبقاء صغار اسماك الكارب الشائع (عليقة السيطرة C بدون اضافة، عليقة رقم 1 بإضافة 0.5% مسحوق الثوم، عليقة رقم 2 بإضافة 1.0% مسحوق الثوم، عليقة رقم 3 بإضافة 1.5% مسحوق الثوم). اظهرت نتائج التجربة الحالية بان اعلى نسبة بقاء 98.7% كانت في معاملة السيطرة وأدنى نسبة بقاء 96.0% في المعاملة الثالثة، بينما اعلى معدل نمو يومي 3744. غم/يوم تحصل من اسماك المعاملة الثالثة واقل معدل نمو يومي 0.3320 غم/يوم لأسماك المعاملة الثانية. ان معدل التحويل الغذائي هي 2.74، 2.85، 2.82، 2.38 لمعاملة السيطرة ومعاملة 1و 2 و3على التوالي. اثبت التحليل الاحصائي للنتائج عدم وجود فروق معنوية P>0.05 في معلات البقاء ومعدلات التحويل الغذائي وكذلك معايير النمو بين معاملة السيطرة والمعاملات الثلاثة وكذلك بين المعاملات الثلاثة ايضا. كان نمط النمو لصغار

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الكارب الشائع قبل التجربة غير متماثل سلبي وان الانحدار b يساوي 2.6496، بينما كان غير متماثل ايجابي لكل المعاملات بعد التجربة. لا توجد اختلافات معنوية $P>0.05$ في معامل الحالة النسبي بين الاسماك قبل التجربة وبعدها وكذلك بين جميع المعاملات.

الكلمات المفتاحية: الكارب الشائع، مسحوق الثوم، معدل النمو اليومي، نمط النمو

Introduction

Common carp, *Cyprinus carpio* is one of the most ancient and famous species that play significant role in freshwater fish production. For this reason, many researchers (Vilizzi *et al*., 2015; Khan *et al.*, 2016; Ljubojević *et al*., 2016) mentioned that this fish introduced in different regions around the world. The production of cultivated fishes at 2020 was (5791.5, 4896.6, 4407.2 and 4236.3) thousand tones for grass carp, *Ctenopharyngodon idella*, silver carp, *Hypophthalmichthys molitrix*, Nile tilapia, *Oreochromis niloticus* and common carp respectively (FAO, 2022). Till now Iraq fish production don't include within world production and the production of common carp is much lower than other countries.

Dabrowskii *et al*., (1984) stated that great attention was given to replace *Artemia* nauplii as food for common carp by a more practical inert diet. In recent years' beneficent microorganisms were added to the feeds of cultivated animals in order to accelerate their growth and enhance their health (Ige, 2013; Nawachi, 2013; Bajagai *et al.*, 2016). These microorganisms were called probiotics. Hutkins *et al*., (2016) pointed out that complex indigestible saccharides added to the feeds in order to accelerate the growth and enhance the health of cultivated animals. These saccharides were called prebiotics, while synbiotics are using probiotics together with prebiotics in the feeds of cultivated animals.

During recent years the attention of general health improvement for cultivated fish was increased throw natural solution that mean using natural substances instead of chemical substances (Hien, *et al*., 2017). In aquaculture the first study on prebiotics done by Hanley *et al*., (1995), while Yazawa *et al.*, (1978) add prebiotics (Many carbohydrate compounds) for the first time in the diets of mammals. Many recent studies deal with promotion of useful bacteria in the alimentary canal by using probiotic or prebiotic and sometime using both of them (Synbiotics) (Lauzon *et al*., 2014; Ringø *et al*., 2014). Mason (2001) stated that many feeds that had oligosaccharides and polysaccharides such as garlic, onion, barley and wheat can be added to fish feed as prebiotics. Many researchers (Mahious and Ollevier, 2005; Bilen and Bilen, 2012; Guerreiro *et al*., 2016) pointed out that the complex saccharides found in some medical plants can enhance fish immunity and improve fish health.

Many studies in Iraq deal with effects of different prebiotics on growth and health of common carp. Al-Atabi (2012) studied the effects of using garlic and ginger on growth parameters and health status of common carp. Ahmed (2014) studied the effects of probiotic (*Saccharomyces cereviciae*), Prebiotic (Fructooligosaccharide) and their combination on growth performance and some blood indices of young common carp. Al-Faiz *et al*., (2014) studied the effects of different levels of garlic powder on some blood parameters of common carp. Al-Faragi (2014) studied the efficacy of prebiotic (*β*-Glucan) as feed additive against toxicity of aflatoxin B1 in common carp. Mustafa *et al*., (2014) investigated influence of chitosan on immune status and survival rate of common carp challenged with bacteria *Aeromonas hydrophila*. Abdulrahman and Ahmed (2015) compare the effects of probiotic (*Saccharomyces cerevisiae*), prebiotic (Fructooligosaccharide FOS) and their combination on white blood cells of young common carp. Abdulrahman *et al*., (2016) studied the effects of FOS on some blood indices of young common carp. Al-Muslimawi and Al-Shawi (2016) studied the effects of addition L-carnitine and niacin on some blood parameters of fry common carp. Mohammad (2016) studied effects of different methods for using common vetch seeds on common carp growth. Taher *et al*., (2018) investigated effects of

addition different levels of bay laurel extract on growth and feed conversion of common carp. All previous studies conducted into fish laboratories, while present experiment aims to study the effects of addition different levels of garlic as prebiotic in the diets on growth and survival of young common carp cultivated in earthen ponds.

Materials and Methods

The current experiment was conducted in earthen ponds at Agricultural Research Station belong to Aquaculture Unit- Agriculture College at Basrah University, Al-Hartha District about 16 km northern-east of Basrah Governorate from 7th August to 21th November 2022. Eight small earthen ponds (20×30×1.5) m were used for current experiment, and each pond stocked with 1500 young common carp (calculated manually) at average weight of 13.2 g. Current experiment conducted to investigate the differences in survival rate and growth performance for these young fishes fed diets with different ratio of garlic meal as additives [without additives (C), addition of 0.5% garlic (T1), addition of 1% garlic (T2), addition of 1.5% garlic (T3)]. The diets were manufactured by Agricultural Consultant Office belonging to Agriculture College using different ingredients (Table, 1).

Total weight and length of fishes were measured at the beginning and at the end of the experiment, while subsamples (cached by seine net) of fishes were weighed periodically and daily feed changed after each weighing. Daily feed was divided into two meals, the first given early on the morning and the second at mid-day. Temperature, pH and salinity of the water for ponds were measured at each sampling period. Throughout this period, six sampling data were collected to calculate the following equations:

Weight increments (WI, g) = FW – IW

Daily growth rate (DGR, g/day) = FW – IW / days

Specific growth rate (SGR, %/day) = 100 * [(ln FW) - (ln IW)] / days

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

Length-weight relationship and condition factor were calculated for fishes at the end of the experiment for each treatment. The following equation was used to calculate the length-weight relationship:

$W= aL^b$ (Pauly, 1983).

Where W= weight of fish in g, L= Length of fish in cm, a = describe the rate of change in weight with length (intercept), and b = weight at unit length (slope).

The condition factors (K) of the common carp were estimated using the following equations:

1- Fulton's condition factor, the value of K was calculated according to Froese (2006):

$K3 = 100\ w/L^3$

2- Modified condition factor (Ricker, 1975) was estimated following Gomiero and Braga (2005):

$Kb = 100\ w/L^b$

3- Relative condition factor 'Kn' (Le Cren, 1951) was estimated following Sheikh *et al*., (2017):

Kn = W/ ^w

Where W= the actual total weight of the fish in g, ^w= the expected weight from length-weight equation formula. The results of current experiment were conducted with a completely randomized design, and the differences between the means were tested by analysis of variance (ANOVA) and the significant differences were tested by LSD test at 0.5% probability level by SPSS program Ver. 26.

(Table 1) Different ingredients with their ration at different treatments.

Feed ingredients	Treatments			
	Control	T1	T2	T3
Fish meal (%)	45	45	45	45
Wheat meal (%)	25	25	25	25
Wheat bran (%)	18	17.5	17	16.5
Barley meal (%)	10	10	10	10
Premix (%)	2	2	2	2
Garlic meal (%)	0	0.5	1	1.5

Results

Table (2) show the average fish weight during the experiment with some environmental factors. Water temperature ranged between 20-30 C^{o}, pH ranged between 7.7-8.0, while salinity ranged between 4.8-7.8 PSU. Highest average final weight (58.7 g) was reached by fishes in pond 3, while lowest final average weight (42.8 g) was reached by fishes reared in pond 4.

Table (3) showed the survival rate and growth criteria of young common carp fed diets with different levels of garlic as additives. Highest survival rate (97.8%) was achieved by fish fed on diet without additives (C) and lowest survival rate (96.0%) was achieved by fish fed on diet with 1.5% garlic as additive (T3). Final average weights of fishes were (51.0, 50.7, 47.6 and 52.1) g for C, T1, T2, and T3 respectively. Highest weight increments (39.7 g) was achieved by fish fed on diet with 1.5% garlic and lowest weight gain (35.2 g) was achieved by fish fed on diet with 1.0% garlic. Highest daily growth rate (0.3744 g/day) was achieved by fish fed on diet with 1.5% garlic and lowest daily growth rate (0.3320 g/day) was achieved by fish fed on diet with 1.0% garlic. Specific growth rate of fishes in different treatments were (1.1643, 1.2732, 1.2885 and 1.3685) %/day for C, T1, T2, and T3 respectively. Feed conversions of all treatment were 2.74, 2.85, 2.82 and 2.38 for C, T1, T2, and T3 respectively. Statistical analysis of the results for survival rate, feed conversion rate and all growth criteria studied in current experiment proved that there were no significant differences ($P>0.05$) between control and other three treatments and also between these treatments.

Table (4) show the averages and ranges of length and weight for young common carp at the end of experiment. Highest average length (12.7 cm) reached by C and lowest (11.5 cm) reached by T3. Table (5) show the parameters of length-weight relationship of young common carp at the beginning of the experiment and for different treatments at end of the experiment. The growth pattern for young common carp before the experiment was negative allometric were b value was 2.6496, while it was positive allometric for all treatments after experiment, where highest slope value (b) was 3.5532 for T2, while lowest value was 3.0028 for T1. The Statistical analysis of the results appeared significant differences ($P\leq0.01$) between slope value and the number three in T2, while there were no significant differences ($P>0.05$) in C, T1 and T3.

Table (6) show the condition factors of young common carp before and after the experiment. The modified condition factor before the experiment was 1.8141, while after experiment were 0.7006, 1.0598, 0.1931 and 0.6723 for C, T1, T2, and T3 respectively. There are no significant differences ($P>0.05$) between relative condition factor before and after the experiment, where its values ranged between 1.0035 in T3 and 1.0161 in T2. Statistical analysis of condition factors results proved that there were significant differences ($P\leq0.05$) in Kb between the beginning of the experiment and the end of experiment and also between the treatment except T3 with C. For

relative condition factor there were no significant differences (P>0.05) between before and after the experiment and also between all treatments. The results of Fulton's condition factor appeared significant differences (P≤0.05) between before and after the experiment, while there were no significant differences (P>0.05) between C with T2 and also betweenT1 with T3.

(Table 2) Measurements of average fish weight during the experiment with environmental parameters.

Date	Average Fish Weight (g)								Temp. (C^o)	pH	Sal. (PSU)
	CP1	CP2	T1P3	T1P4	T2P5	T2P6	T3P7	T3P8			
7/8/2022	16.3 ±13.9	13.3 ±11.9	12.7 ±10.2	13.3 ±9.8	9.6 ±10.9	15.3 ±13.9	14.6 ±10.2	10.2 ±9.9	30	7.7	6.5
29/8	23.3 ±13.1	17.9 ±13.9	23.7 ±12.9	18.7 ±13.7	15.0 ±13.9	21.9 ±15.9	13.4 ±9.9	15.3 ±12.0	28	7.7	7.0
19/9	26.4 ±15.3	19.4 ±13.3	26.3 ±12.3	21.8 ±12.8	17.5 ±15.9	25.2 ±23.7	22.8 ±14.4	18.2 ±11.5	25	7.8	7.8
10/10	43.3 ±19.1	28.4 ±15.9	39.5 ±19.9	27.1 ±15.9	30.1 ±19.4	34.3 ±29.8	30.9 ±16.4	25.0 ±14.0	25	8.0	7.0
31/10	46.7 ±20.9	34.5 ±17.2	40.7 ±24.8	31.8 ±22.1	32.0 ±21.9	42.7 ±33.6	45.8 ±22.2	33.7 ±16.9	23	8.0	6.5
21/11	57.7 ±26.6	44.4 ±20.9	58.7 ±37.9	42.8 ±28.9	43.3 ±28.3	52.0 ±40.0	54.6 ±23.9	49.6 ±20.1	20	7.8	4.8

(Table 3) Survival rate and growth criteria of different treatments in the experiment.

Growth Criteria	Control		T1 (0.5%)		T2 (1%)		T3 (1.5%)	
	P1	P2	P3	P4	P5	P6	P7	P8
SR (%)	98.1	97.6	92.9	100	92.7	100	94.1	98.0
Average	**97.8 a**		**96.4 a**		**96.3 a**		**96.0 a**	
FW (g)	57.7	44.4	58.7	42.8	43.3	52.0	54.6	49.6
Average	**51.0 a**		**50.7 a**		**47.6 a**		**52.1 a**	
WI (g)	41.4	31.1	45.9	29.5	33.7	36.7	40	39.4
Average	**36.2 a**		**37.7 a**		**35.2 a**		**39.7 a**	
DGR (g/day)	0.3904	0.2943	0.4340	0.2787	0.3180	0.3460	0.3770	0.3718
Average	**0.3423 a**		**0.3563 a**		**0.3320 a**		**0.3744 a**	
SGR (%/day)	1.1914	1.1372	1.4410	1.1055	1.4250	1.1520	1.244	1.4930
Average	**1.1643 a**		**1.2732 a**		**1.2885 a**		**1.3685 a**	
FCR	2.54	2.95	2.67	3.03	2.64	3.01	2.67	2.10
Average	**2.74 a**		**2.85 a**		**2.82 a**		**2.38 a**	

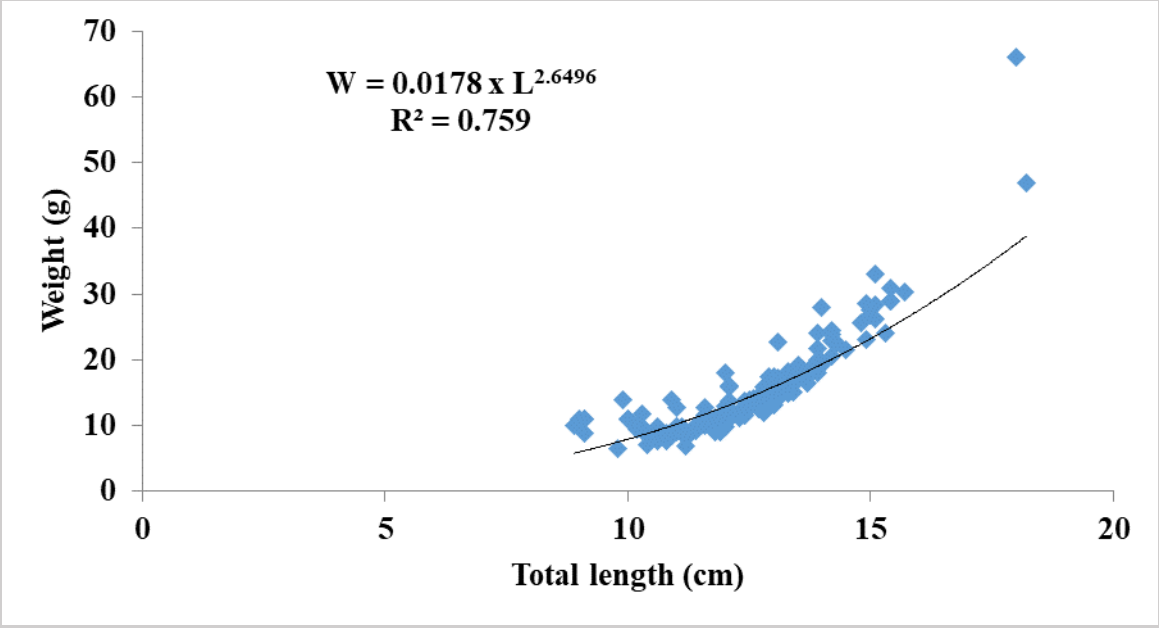
Different letters in one row is significantly different (P≤0.05).

(Table 4) Data on length and weight of young common carp at end of the experiment.

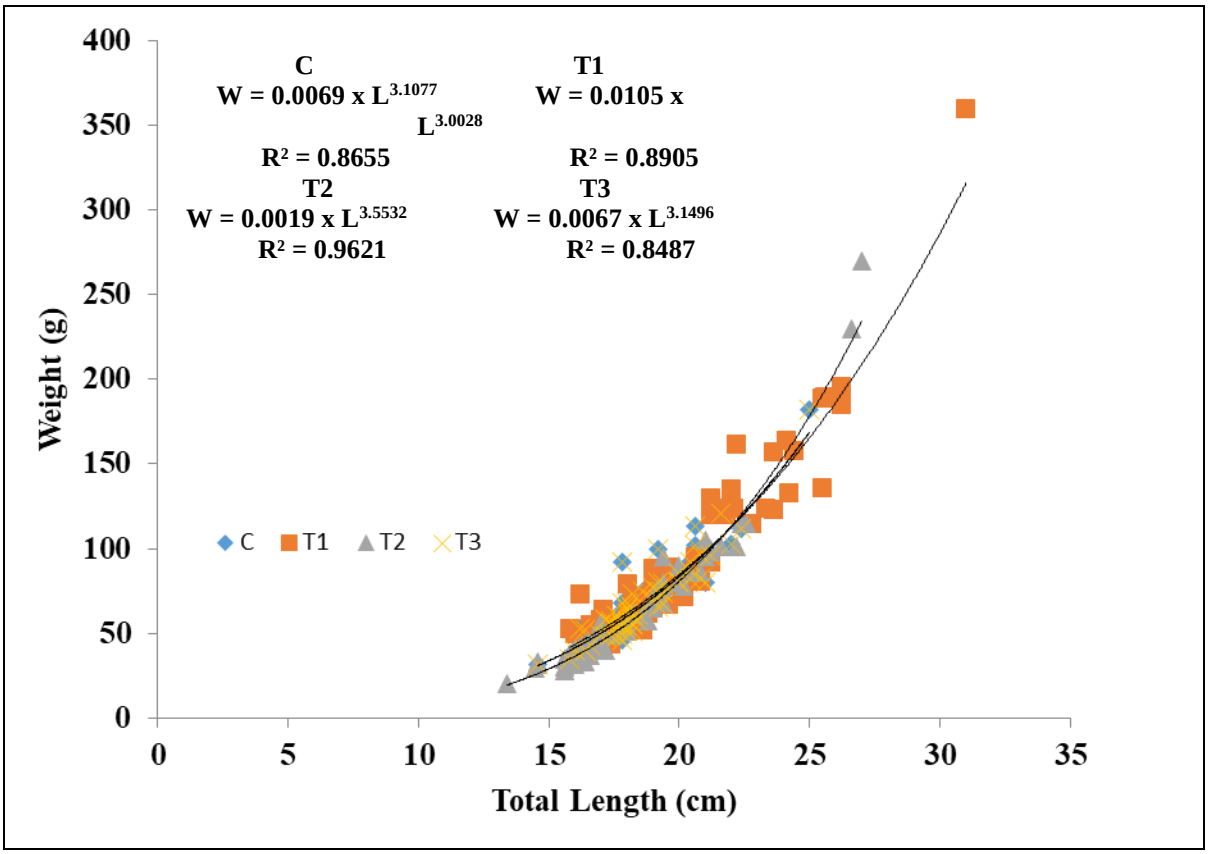
Treatments	Length range (cm)	Weight range (g)	Mean length (cm)	Mean Weight (g)
C	14.0-25.6	22.0-169	18.3	51.0
T1	15.8-26.2	32.0-196.0	19.3	50.7
T2	13.4-27.0	20.0-270.0	17.9	47.6
T3	14.6-25.0	32.0-182.0	18.6	52.1

(Table 5) Equation parameters of length-weight for young common before and after the experiment.

Treatments	a	b	R^2	t value (calculated)	Significance of t
Before Experiment	0.0178	2.6496	0.7590	-2.0215	0.0903
After Experiment					
C	0.0069	3.1077	0.8655	0.2779	0.3909
T1	0.0105	3.0028	0.8905	0.0051	0.4979
T2	0.0019	3.5532	0.9621	1.6718*	0.0495
T3	0.0067	3.1496	0.8487	0.2364	0.4069



(Figure 1) Length-weight relationship for young common carp before the experiment.



(Figure 2) Length-weight relationship for four treatments of young common carp at end of the experiment.

(Table 6) Condition factors of young common carp after the experiment.

Treatments	Condition factors		
	Modified condition factor Kb= 100 W/ Lb	Relative condition factor Kn= W/ W^	Fulton's condition factor K3= 100 W/ L3
Before Experiment	1.8141 a ±0.3939	1.0072 a ±0.2493	0.7524 d ±0.1779
After Experiment			
C	0.7006 c ±0.0938	1.0154 a ±0.1359	0.9574 c ±0.1277
T1	1.0598 b ±0.1529	1.0093 a ±0.1457	1.0687 a ±0.1542
T2	0.1931 d ±0.0185	1.0161 a ±0.0974	0.9516 c ±0.1169
T3	0.6723 c ±0.0874	1.0035 a ±0.1305	1.0403 a ±0.1357

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

Discussion

Gatesoupe (2005) referred that prebiotics is another effective way of disease control in aquaculture, and it was food ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract. Ringø *et al*., (2010) and Merrifild *et al*., (2010) stated that probiotic and prebiotics were added to the diets of fishes in order to increasing fish immunity and fish growth by many processes such as stimulate fish appetite, helping in digesting some complex compounds and improvement of feeds by production of vitamins and enzymes. Results of current experiments proved that there were no effects for adding different levels of garlic to the feed of young common carp which cultivated in earthen ponds, where there were no significant differences ($P>0.05$) in growth, survival and feed conversion between control and other three treatments. This results can be attributed to the availability of natural foods found in earthen ponds consumed by these young fishes. Olsen *et al*., (2001) referred that the benefits of adding prebiotics depend on the ability or inability of microorganisms to leavening additional quantities of prebiotics, while Venter (2007) recorded that the continuous adding of prebiotics may lead to medications of some diseases microorganisms to get benefits from the carbohydrates found in some prebiotics. Al-Asha'ab *et al*., (2014) mentioned that the supplementation of 5 g FOS per kg feed hadn't any effects on growth of young common carp. Gatlin 111and Li (2004) pointed out that the supplement of Grobiotic-A to the diet of hybrid striped bass enhance growth and diseases resistance. Mazurkiewicz *et al*., (2008) stated that during a 50-day growth test, the common carp receiving Fermacto prebiotic feeds showed significantly ($P\leq0.05$) higher mean individual body weight in comparison with the control group.

The results of current experiment are differ too much from the results of other studies in Iraq because most of these studies conducted inside fish laboratories. The addition of FOS to the diets of young common carp improve growth criteria (Ahmed, 2014; Abdulrahman and Ahmed, 2015). The growth parameters of common carp juveniles were improved at feeding on diet supplemented with some organic acids (Muhsan and Al-Shawi, 2016). Taher *et al.*, (2018) stated that the addition of 2% bay laurel's (*Laurus nobilis*) leaf extract to the diets of common carp fingerlings improve growth criteria and feed conversion rate, where weight increment of 7.63 g achieved compared with 5.42 g in fingerlings fed diets without addition (control), and also better feed conversion rate was 4.56 compared with 6.59 for control. The results of current experiment are resemble to the result of Taher *et al.*, (2024) who investigate the effect of adding commercial probiotic, prebiotic (Onion) and both of them on growth and survival of juvenile common carp cultivated in earthen ponds.

It is well known that relative condition factor was consider the best for cultured species comparing with modified and Fulton's condition factors, so the results of condition factors in current experiment revealed that there are too much differences in the modified and Fulton's condition factors with very little differences in relative condition factor. It is well known that condition factor gives clear picture about growth of fishes and it is dependent on the growth in nature and also in cultivation conditions, for this reason it is clear that the addition of different ratio of garlic doesn't affect the condition factors of young common carp in current experiment.

Conclusion

The results of current study revealed that there were no significant effects of adding garlic as prebiotic to the feeds of young common carp on survival rate, conversion rate and all growth criteria studied.

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