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Effect of Omega-3 Fatty Acids (EPA / DHA) and Vitamin E Addition on the Growth Performance and the Larvae Performance of *Cyprinus carpio* L.

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Abstract: This study was conducted in earthen ponds of shrimp hatchery belonging to the Basrah Agricultural Directorate, to find out the effect using of food additives omega-3 and Vitamin E on the performance of the growth and the quality of the produced larvae of the common carp *Cyprinus carpio* L.. Three treatments were used, the first treatment (T1) represented control without any food additives, the second treatment (T2) contained 5gm.Kg^{-1} diet of omega-3 fatty acids and the third treatment (T3) contained Vit. E was added at 200 mg.Kg^{-1} diet. The experiment lasted for 82 days. For each treatment three replicate were used and six common carp brood stock in each replicate. The productive parameters (body weight, weight gain, feed conversion rate, feed efficiency ratio% and specific and relative growth rate%) were measured. Results indicated an improvement in all production parameters and in the feed conversion efficiency of for T2 and T3, compared with the T1. The weight gain (526.39 gm.fish^{-1}), feed conversion rate was 3.61 and feed efficiency ratio was 28.13% for the T2 which showed a significant differences ($P<0.05$) compared to the control, while T3 did not show significant differences ($P>0.05$) in comparison with both treatments T1 and T2; the specific and relative growth rate for T2 were 0.32%.day^{-1} and 30.81 %.day^{-1} respectively showed also a significant differences ($P<0.05$) compared with the control. These results showed that addition Vit. E to the diet improved the production parameters of common carp larvae better than in omega-3 and control treatments. The study concluded that addition omega-3 fatty acids at a concentration of 5gm Kg $^{-1}$, and Vit. E at a concentration of 200 mg kg^{-1} to the diet enhanced the growth rates of common carp and improved larvae production.

Key words: *Cyprinus carpio*, common carp, omega-3 fatty acids (EPA / DHA), Vitamin E.

Introduction

Fishes represent about 17% of the animal protein consumed by the world's population. Moreover, fish provided for about 3.2 billion people, and provided approximately 20% of the average per capita animal protein intake; fishes is a very nutritional source and especially helps to compensation the nutrient deficiency (FAO, 2018). Scientific reports indicated that the functions of essential fatty acids of the two groups i.e. omega-3 and omega-6 are fluctuated after knowing that high doses of essential fatty acids from omega-6 have some risks affecting health when the ratio increased from 1:1 during development to 20:1 at present or even higher (Simopoulos, 2016). Increasing in omega-6 intake include cancerous tumors, formation of self-thrombosis, and the response to infections, so recommendations for increasing n-3 and reducing n-6 consumption have been proposed by nutritionists (Zhang *et al*., 2020). Health benefits of essential omega-3 fatty acids, especially Docosahexaenic (DHA, C22:6 n-3) and Eicosapenaenic acid (EPA, C20:5 n-3) which found in fish oil and alpha-linolenic acid (ALA, C18:3 n-3) that found in vegetable oils contradict the harmful effects of omega-6 fatty acids (Burdge *et al*., 2002). Omega-3 was considered an essential fatty acid for humans as final consumer of fish (Ramezani *et al*., 2012).

Determining the exact amount of requirement of essential fatty acids is difficult because it depends on the quality and source of fat, omega-3/omega-6 ratio in food and fatty acid metabolism in the body (Bezard *et al*., 1994). The dietary important of the highly unsaturated fatty acids (HUFA) for marine fish was known earlier (Sargent *et al*., 1997).

Vit. E is one of the most important non-enzymatic soluble antioxidants in fat, as it works to remove the oxygen root and thus is the first line in removing fat oxidation (Puangkaew *et al*., 2005). Naturally it consists of from alpha-tocopherol, beta-tocopherol, delta-tocopherol, Kama-tocopherol, as well as alpha-tocotrienol, beta-tocotrienzole, delta-tocotrienol (Sen *et al*., 2006); researchers indicated the importance of Vit. E in improving the immune response of fish, as it modifies the physiological changes of rainbow trout Oncorhynchus mykiss when fed on diets containing high concentrations of unsaturated fat (Puangkaew *et al*., 2005). Frischknecht *et al*. (1994) found that a deficiency of Vit. E in the diet of rainbow trout leads to poor growth.

The aim of the present study was to evaluate the effect of omega-3 fatty acids (EPA/DHA) and Vit. E on growth performance and the larvae quality of common carp Cyprinus carpio.

Materials & Methods

Study site

The research work was implemented for all experiments in shrimp hatchery belonging to the Basrah Agricultural Directorate for the period from 29th October.2018 to 17th July 2019. The study site is located in the northern Basrah Governorate, 21 km from the city Centre. The hatchery area is one hectare consisting of four earthen ponds, (20 × 80 m) and depth of 1.5 m. In addition, it contains a hatching hall with an area of 8 ×12 m.

Fish of the experiment

A total of 54 individuals of common carp brood stock were used in all study experiments, since it considered the primary breeding fish achieved in Iraq, brought from a local floating cages fish farms in Al-Qurnah city, Fishes were transported to the study site by means of pickup. Fishes were sterilized by saline solution (5% NaCl) for 5-10 minutes as soon as they arrived at the work site to get rid of fungi and other pathogenic organisms that may stuck on the skin or gills.

The larvae were obtained from the reproduction of the same fishes used in the growth experiment. For each treatment 10,000 larvae were stocked in triplicate at the beginning of the experiment. After experiment completion, the pond was emptied and all the larvae were caught to measure weight and to calculate the survival rate.

Design of the experiment

The experiment was designed with three treatments (control T1, omega-3 T2 and Vit. E T3) each has three replications, six individuals for each replication. Nine cages that made from polypropylene random copolymer (PPR pipe) material were manufactured for the three treatments (length ×width × height 3 ×1.7 × 1.8 m), surrounded by nets with 10 × 10 mm mesh size, to isolate the three treatments and easy monitoring and feeding of the fish. These cages were placed inside the earthen ponds which filled with water to one meter height. The fish were randomly distributed in nine cages made by PPR. The brood stock was fed with 3% body weight (BW), two times a day, one at 8 AM and the second at 2 PM. The amount of feed was adjusted according to the changing of the biomass every two weeks. This experiment continued from 28 October 2018 to 30 March 2019.

The second experiment carried out in earthen ponds to cultivate the produced larvae with an area of 80 x 10 x 1.5 m, as the ponds were prepared by removing the sediments and plants. Every pond was divided into three treatments, each with three replication in an area of 9 ×10 × 1.5 m and separated by iron pegs and divided by fine mesh. This experiment continued for 62 days from 28 April to 23 July 2019.

Experimental diets:

The diets that used in the experiments were prepared after calculating the percentages of each feed component and mixing it for homogeneity. The experimental diets (the three treatments) were manufactured in the Feed factory in the College of Agriculture, University of Basra, as shown in table (1), the chemical analysis of the diets were carried out in the quality control laboratory of the Animal Resources Department of the Ministry of Agriculture.

Table (1): Ingredients and chemical analysis of the experimental diets.

Ingredients	Control (T1)		Omega -3 (T2)		Vit. E (T3)	
	Brood stock	Larvae	Brood stock	Larvae	Brood stock	Larvae
Fish meal	30	35	30	35	30	35
Wheat flour	45	40	45	40	45	40
Wheat bran	23	25	23	25	23	25
Sunflower oil	2	2	2	2	2	2
*Omega -3 ($g.Kg^{-1}$)	0	0	5	5	0	0
Vit. E ($mg.Kg^{1}$)	0	0	0	0	200	200
Chemical analysis (%) as DM						
Moisture	10.1	10.1	9.7	9.5	8.8	9.4
Crud protein	21.9	22.7	23.3	25.8	22.8	23.8
Crud lipid	4.1	4.9	5.5	6.7	4.8	5.4
Crud fibre	4.6	5.8	4.1	6.6	3.9	6.9
Nitrogen Free Extract (NFE)	59.3	56.5	57.4	51.4	59.7	54.5

*(EPA and DHA)

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Productive parameters

The following equations were used to describe the growth performance of brood stock:

Weight gain:

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

Relative Growth Rate:

$$\text{RGR} = \frac{(\text{W2 g}.fish^{-1} - \text{W1 g}.fish^{-1})}{W1} \times 100$$

Specific Growth Rate:

$$\text{SGR} = \frac{(\ln\text{W2 g}.fish^{-1} - \ln\text{W1 g}.fish^{-1})}{T1 - T2} \times 100$$

Where lnw2 is the natural logarithm of the final weight at the time T2, lnw1 is the natural logarithm of the initial weight at the time T1and T2-T1 is the period between the two weights.

1- Feed Conversion Rate:

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

2- Feed Efficiency Rate:

$$\text{FER} = \frac{WG}{R}$$

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

3- Survival rate:

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

For fish larvae all the above parameters were also used, but RGR was modified, due to the large differences between initial and final weight (Myszkowski, 1997) as follow:

$$RBR = 100(\, e^{\frac{SGR}{100}} - 1)$$

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 fibre

Statistical analysis:

All parameters were tested using one-way analysis of variance (ANOVA). Significant results (P<0.05) were tested using LSD to test significant difference between means. This statistical analysis was completed with the computer software SPSS package.

Results

In the first experiment where fish reared in three treatments (control, omega-3 fatty acid group, Vit. E) with an average initial individual weight (1476.74, 1699.17 and 1723.06 g.fish^{-1}) respectively. It was noticed in table (2) that the average of final weight of the T2 (omega-3) were higher than T1 but there was no significant differences (P>0.05) between them. It was found that from the statistical analysis refers to a significant differences (P<0.05) in the total and daily weight showing existing increase between treatment T1 and T2 which were 287.23 and 526.39 g.fish^{-1} respectively, while it was not significant differences (P> 0.05) between treatment T3 and both other treatments (Table 2).

Results indicated that the specific and relative growth rates of the T1 was 0.21 %. d^{-1} and 19.09% respectively, while for the T2 it was 0.33%.d^{-1} and 30.82 % respectively and 30.82% and for treatment T3 recorded 0.25 % . d^{-1} and 22.70% respectively. Results of using omega-3 (T2) showed a significant differences (P<0.05) for the specific growth rate and the relative growth rate with the T1 and T3 treatments, while statistical analysis showed that there was no significant differences (P>0.05) between the T1 and T3 (Table 2).

Treatment T1 recorded 5.58 in feed conversion rate and 18.37% in feed efficiency ratio, while in T2 it was 3.61 and 28.13% for food conversation and food efficiency respectively and, treatment T3 reached 4.5 in food conversion rate and 22.96% in a food efficiency ratio. The statistical analysis showed significant differences (P<0.05) between treatment T1 and T2 but no significant differences (P>0.05) between T3 with other two treatments (Table 2).

For larvae, the experiment was conducted to compare between T1 control and the addition of the omega-3 (T2) fatty acid group and Vit. E (T3) in the diets of common carp larvae that reared in earthen ponds and their effect on growth performance and survival rate.

Table (2): Productive parameters of common carp stock fed on three experimental diets (Mean ± SD).

Parameters	Treatments		
	Control (T1)	Omega-3 (T2)	Vitamin E (T3)
IW (g)	1476.74 a ±193.58	1699.17 a ±350.99	1723.06 a ±168.15
FW (g)	1763.97 a ±288.56	2225.56 a ±47.10	2119.17 a ±279.47
WG (g)	287.23 a ±95.01	526.39 b ±126.61	396.11 ab ±111.68
DWG ($g.d^{-1}$)	3.50 a ±1.59	6.42 b 1.54±26.	4.83 ab ±1.36
S G R ($\%.d^{-1}$)	0.21 a ±0.04	0.33 b ±0.01264	0.25 ab ±0.04
R G R %	19.09 a ±4.17	30.82 b ±1.35625	22.70 ab ±4.49
F C R	5.58 a ±1.12	3.61 b ±0.55895	4.52 ab ±1.05
F E R %	18.37 a ±3.50	28.137 b ±4.02	22.97 ab ±5.44

Data with different superscripts are significantly different (P<0.05)

Table (3) shows the initial, final weight of common carp larvae fed on two food additives. No significant differences (P>0.05) among the three treatments were noticed. While the results of the statistical analysis of the total and daily weight gains showed significant differences (P<0.05) among the three treatments in daily weight gain only. The specific growth rate showed significant differences (P<0.05) among the three treatments, but only significant differences (P<0.05) between T3 and other two treatments was obvious for relative biomass growth rate. In all above parameters, T3 exhibited high values.

Feed conversion rate and food efficiency ratio of control and the food additives diets showed in table (3), by 5.15 and 19.48% respectively in T1, and 3.33 and 32.25 respectively in T2 and % and 3.72 and 28.47% respectively in T3. The statistical analysis of the feed conversion rate and the feed efficiency ratio showed no significant differences (P>0.05) among the three treatments .

Survival rate was 3.77 % for T1 and 23.77 % for T2 but there was no significant differences (P> 0.05), While T3 recorded highest survival rate (23.77%), which differ significantly (P< 0.05) with the other two treatments.

Table (3) Productive parameters of common carp larvae fed on three experimental diets (Mean ± SD).

Parameters	Treatments		
	Control (T1)	Omega-3 (T2)	vitamin E (T3)
IW (mg)	57.59 a ±21.77	169.02 a ±16.48	20.51 a ±2.10
FW (mg)	6254.76 a ±171.14	3745.24 a ±1168.73	8976.54 a ±5101.33
WG (mg)	6197.17 a ±159.40	3576.22 a ±1152.37	8956.03 a ±5099.40
DWG ($mg.d^{-1}$)	108.72 a ±2.80	62.74 b ±20.23	157.12 c ±89.46
S G R ($\%.d^{-1}$)	8.30 a ±0.59	5.38 b ±0.37	10.50 c ±0.76
R B R %	133.78 a ±67.72	149.09 a ±87.03	384.40 b ±93.40
F C R	5.15 a ±0.401	3.34 a ±1.059	3.73 a ±1.102
F E R %	19.49 a ±1.462	32.25 a ±11.053	28.47 a ±9.479
Survival %	3.77 a ±2.011	23.77 b ±13.876	13.78 a ±13.068

Data with different superscripts are significantly different (P<0.05)

Discussion

The total weight increase was recorded in all the used treatments, while the treatment T2 (fish fed on a diet containing omega-3) showed the highest rate of total and daily weight gain compared with vitamin E addition and control, Al-Dubakel *et al*.(2012) use Roquette oil as an additive in the diet of common carp young's and recorded high weight increase due to this addition . It was clear from the above results that the best weight increase achieved when omega-3 offered under the conditions of rearing in the earthen ponds. The least weight increase was recorded from the treatment T1. The use of omega-3 seems to have improved the nutritional value of the experiment diet. Dietary lipid is more important energy source than carbohydrate in feeding of carnivorous fish and has sparing function on dietary protein (Lee *et al*., 2002). Perhaps the reason is that lipase is more effective than amylase also fat have the effect of providing protein for growth superior than carbohydrates. Madsen *et al*. (1999) showed that DHA performs many important functions in fish, including metabolism, and that its presence in cellular membranes regulates the integrity and function of the membrane. Taşbozan & Gokce (2017) pointed that studies on freshwater fish have shown that their n-3 EFA requirements are mostly focused on linolenic acid (18:3n-3). Consuming enough fatty acids ensures not only that immune cells were built robustly, but also that they work more effectively as well (Copeman *et al*., 2002).

In an experiment conducted by Tidwell & Robinette (1990) who added palm oil as a source of omega-6 and fish oil as a source of omega-3 in channel catfish *Ictalurus punctatus* diets at 1.5% and 1% respectively and achieved similar values in the overall weight increase. This was indicated by many studies (Bogut *et al*. 2002; Ji *et al*., 2009; Al-Souti *et al*., 2012), who emphasized that diets containing polyunsaturated fatty acids (PUFA) will inhibit the enzymatic activity in Lipogenesis (the metabolism process by which the acetyl-COA is converted into triglycerides and stored in fats), or stored fat in the liver and consequently the fatty acids had a positive impact in the defense of anti-oxidant systems in fishes. However, it has been noted that the absorption of polyunsaturated fatty acids (PUFAs) in fish diet (especially EPA and DHA) will increase the oxidative stress (OS) which may affects normal physiological function (Jin *et al*., 2017). on the other hand ALA α-linolenic acid (ALA, C18:3n-3) has an important physiological function, since it is an essential fatty acid for freshwater fish (Yu *et al*., 2019).

The needs of common carp and grass carp from omega-6 and omega-3 were studied, it was found that grass carp when fed on diets without essential fatty acids showing symptoms of deficiency, including curvature of the spine and low growth rates, therefore, the proportion of protein digestibility must be appropriate with the ability to digest fats to achieve a high growth rate and efficiency in feed conversion rate (Takeuchi *et al*., 1980; Yang *et al*., 2008; Xu *et al*., 2017).

Results also indicated that the addition of Vit. E had an important role in enhancing rates of total and daily weight gain of common carp, the reason may be in strengthening the antioxidant regimen as the main function of Vit. E is to prevent the formation of peroxides for unsaturated fatty acids and thus protect against oxidation, especially the membranes of fat cells, which gives more vitality for the functioning of a living cells (Sharifzadeh *et al*., 2015). As unsaturated fatty acids are important structural components of living cells, including cell specificity, fluidity, elasticity, permeability, and efficacy of cell enzyme binding processes (Stillwell & Wassall, 2003). Although adding vitamin E did not differ significantly from control in most parameters, it resulted in a higher weight gain, and this shows its importance in fish diets, Pan *et al*. (2017) indicated that a lack of Vit. E supplementation in the diet leads to a decrease in the growth rates, and weakens the immune function of young grass carp, but adding Vit. E in the ideal proportions to the diet will lead to altering these negative effects.

The requirements of some fish species from Vit. E range between 120 mg.kg-1 diet (Hamre & Lie, 1995) to more than 550 mg.kg-1 (Ruff *et al*., 2003) for performance the optimal growth and disease resistance. However increasing the concentration of vitamin E in a 30% lipid feed from 300 to 1500 mg.kg-1 diet can reduce the rate of lipid oxidation in fish fillets and reduce the formation of off flavours (Chaiyeapechara *et al*., 2003). However some research indicated that adding Vit. E to fish diets does not affect growth performance (Forster *et al*., 1988; Mourente *et al*., 2000; Li *et al*., 2008).

Results of statistical analysis showed that specific and the relative growth rate for the treatment omega-3 differ significantly compared to Vit. E and control treatments. These results are consistent with what Morson & Clandinin (1986) indicated, as there is a biomechanical mechanism in which essential fatty acids may affect metabolic pathways and their ability to act as effects in all activities of other enzymes related to metabolic pathways that has a vital role in improving the growth. Bou *et al*. (2017) indicated that the diet containing PUFA n-3 (0. 2-0.5 %) improved the specific growth rate of Atlantic salmon (*Salmo salar*) compared to fish fed on a PUFA n-3 fatty acid-free diet. Jalali *et al*. (2008) found that final weight, daily growth rate, specific growth rate and weight gain were higher in beluga (*Huso huso*) larvae fed with vitamin E and highly unsaturated fatty acid (HUFA).

Conclusion

It was concluded from the present study that addition of both omega-3 and Vit. E to the diet of common carp enhance the growth of brood stock and increase larval survival rate compared to diets without these additives.

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تأثير اضافة الاحماض الدهنية أوميغا 3 (EPA / DHA) وفيتامين E على أداء النمو وأداء يرقات *Cyprinus carpio* L.

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المستخلص: أجريت هذه الدراسة في الأحواض الترابية في مفقس الروبيان التابع لمديرية زراعة البصرة، لمعرفة تأثير استخدام الاضافات الغذائية أوميغا 3 وفيتامين E على أداء نمو ونوعية اليرقات المنتجة من اسماك الكارب الشائع *Cyprinus carpio* L.. تم استخدام ثلاث معاملات, المعاملة الاولى (T) تمثل السيطرة بدون أي اضافة غذائية والمعاملة الثانية (T2) تحتوي 5 غم لكل كغم من العليقة على الاحماض الدهنية أوميغا 3 والمعاملة الثالثة (T3) تحتوي 200 ملغم لكل كغم من العليقة على فيتامين E. استمرت التجربة لمدة 82 يومًا. استخدم لكل معاملة ثلاث مكررات وستة اسماك من امهات الكارب الشائع في كل مكرر. تم قياس المقاييس الإنتاجية (وزن الجسم والزيادة الوزنية ومعدل التحويل الغذائي ونسبة كفاءة التغذية ومعدل النمو النسبي والنمو النوعي). أشارت النتائج إلى تحسن في جميع مقاييس الإنتاج ومعدل التحويل الغذائي للمعاملات T2وT3، مقارنة مع المعاملة. T1 الزيادة الوزنية (526.39 غم لكل سمكة) و معدل التحويل الغذائي 3.61 ونسبة كفاءة التغذية 28.13٪ للمعاملة T2 والتي أظهرت اختلافات معنوية (P <0.05) مقارنة بمعاملة السيطرة ، بينما لم تظهر المعاملة T3 فروقات معنوية (P> 0.05) مقارنة بكل من المعاملتين T1 و T2. كانت معدلات النمو النوعي والنمو النسبي للمعاملة T2 0.32% لكل يوم و30.81 % على التوالي والتي اظهرت ايضا اختلافات معنوية (P <0.05) مقارنةً بمعاملة السيطرة. أظهرت الدراسة الحالية أن إضافة 3 فيتامين E الى العليقة ادى إلى تحسين معايير إنتاج يرقات الكارب الشائع بشكل أفضل مما هو عليه في حالة اضافة أوميغا 3 ومعاملة السيطرة. يستنتج من الدراسة الحالية إلى أن إضافة الأحماض الدهنية أوميغا 3 بتركيز 5 غم لكل كغم وفيتامين E بتركيز 200 ملغم لكل كغم الى علائق الكارب الشائع يؤدي الى تعزيز معدلات النمو وتحسين إنتاج اليرقات.

الكلمات المفتاحية: *Cyprinus carpio* , الكارب الشائع, الاحماض الدهنية اوميغا-3 (EPA / DHA), فيتامين E.