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Evaluation Of Thepax And Endo Vit. C As Growth Promoters For Common Carp Cyprinus Carpio

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Abstract: The present study aims to evaluate the effect of Thepax and Endo Vit. C as feed additives on the growth performance of common carp Cyprinus carpio in cages earthen pond. A total of 600 common carp fish with an average weight of 163.41±10.16 g were distributed in the cages at a density of 100 fish/cage. Two replications for each treatment were used and three diets were formulated (27.84% crude protein), two diets with additives which included a control T1 (0% additives), T2 (1g/kg Thepax) and T3 (1g/kg Endo Vit. C). The highest value of the final weight was recorded in Thepax treatment (2209.34 g), followed by the Vit.C and the control treatment, and in all growth parameters (WG, DGR, RGR and SGR) statistical analysis showed significant difference (P≤0.05) between Thepax treatment with both Vit. C and control treatments. The survival rate was highest for Vit. C (99.5%), then Thepax (99%) and control treatment (83.5%). Feed utilization showed that the best food conversion ratio (3.14), PER (1.215) for Thepax, while PPV recorded highest value (20.379%) in Vit.C. Condition factor (K), reached the highest value (2.078) for Thepax treatment and the lowest value was for the control treatment. It was noted that body protein content of the Vit. C treatment was superior (17.94%), while it reached 16.10% for Thepax treatment, also the percentage of body lipid was higher after the experiment than the value before the experiment in all treatments. On the basis of the results obtained for above parameters, it was concluded that addition of 1g/kg Thepax in the diet recommended for common carp in earthen pond .

Keywords: Thepax , Additives, PER, PPV, FCR, Endo Vit. C

Introduction

Fish nutrition plays a critical role in aquaculture, as it influences both fish growth and health, and this need high-quality feeds (Singh et al., 2021). Feed costs represent around 80% of the

total operating costs of aquaculture projects (El-Sayed, 2014). Aquaculture production is expected to continue to grow in the future, in response to practical difficulties, high-efficient technologies of feed enzyme and microbial fermentation have been developed, to improve the bioavailability and feed efficiency of raw materials (Zhou et al., 2013; Jasim et al., 2016; Shao et al., 2019; Rahimnejada et al., 2019). At the same time, the supply of fishmeal and fish oil produced from marine resources cannot increase further (Naylor, 2021; Salih et al., 2021).

To achieve sustainable development in aquaculture, feed additives are important tool in aquaculture production that can be used to make production more effective by decrease the need for medicated treatments and waste discharges (Gonçalves & Santos, 2017). Feed additives represent different types of molecules, compounds or organisms that stimulate ingestion, absorption and assimilation of nutrients (Watts et al., 2020). Since feed costs are a major expense in aquaculture production, so the products that improve feed efficiency are particularly important (Nates, 2016), most of these feed additives are non – nutritious and include antibiotics, immunostimulants, antioxidants and probiotics or prebiotic (Dawood et al., 2017; Imran et al., 2019; Kord et al., 2021) which added to improve feed quality and health performance, feeding efficiency of the fishes (Al-Dubakel et al., 2014, FAO, 2016).

Yeast was used as fed additive for many farm animals more than a hundred years ago (Owens & McCracken, 2007) it contains enzymes, amino acids, fatty acids, vitamins and may contain unknown growth factors, in aquaculture feeds only a few species were used, the most common was Saccharomyces cerevisiae (Encarnação, 2016, Agboola et al., 2021). Thepax consider as a probiotic in poultry feeds (Yousefi & Karkoodi, 2007; Zarei et al., 2011; Boostani et al., 2013; Fazli et al., 2008), while other researchers consider it as prebiotic (Nikpiran et al., 2013; Al-Mhanawi et al., 2021), however the manufactured company mention it as prebiotic since it consist from cell wall of yeast Saccharomyces cerevisiae, mannan and glucan (Nikpiran et al., 2013). Thepax support gut health by several possible mechanisms (Cakir et al., 2008). Thepax improve intestinal enzyme and a significant increase in at least 11% in lactic acid bacteria population was observed (Adel et al., 2016), Ke et al. (2021) found that antimicrobial peptides, and herbs can alter the richness, diversity, and composition of the intestinal microbiota of common carp. Al-Dubakel et al. (2015) concluded that the use of local Iraqi probiotic by 2 ‰ achieved the best growth, weight gain and feed conversion rate for common carp juveniles.

Vitamin C (Ascorbic acid) have antioxidant properties, acts as a reducing agent that assist in synthesizing collagen, carnitine, norepinephrine, peptide hormone, and tyrosine metabolism (Harrison & May, 2009; Adeyemi-Doro & Iyiola, 2018) and it was assumed that vitamin C in fish is an essential nutrient for optimum growth, as most species cannot synthesize this vitamin because of the lack of L-gulonolactone enzyme (Ching et al., 2015; Trichet et al., 2015; Adeyemi-Doro & Iyiola, 2018), therefore diet is the main exogenous source, and due to the limited ability of this vitamin to be preserved in the fish, a regular and adequate intake is necessary to prevent deficiency (Luo et al., 2021). Dietary vitamin C requirements have been reported in several cultured fish species, which vary due to the difference in fish species, size, vitamin C form, feed formulations, feeding behavior and culture system (Dabrowski, 2000; NRC, 2011).

The use of unstable forms of vitamin C in the feeds is often lead to overestimation of requirements, while the use of stable forms is the most reliable estimation of requirements (Woodward, 1994; Gouillou-Coustans & Kaushik, 2000), about 75% of vitamin C activity in practical diets is lost during manufacturing and storage due to exposure to high temperature, oxygen and light (Shiau & Hsu, 1993), Riaz et al. (2009) stated that extrusion temperatures effect on vitamin C is destructive and it is almost completely lost during processing.. However, only poor information is available on the requirements of vitamin C as well as other water soluble vitamins, and deficiency have been observed in common carp (Dabrowski et al., 1988; Sándor et al., 2017).

The present study was undertaken to evaluate the effect of Thepax and Endo Vit. C as feed additives on the growth performance of common carp Cyprinus carpio .

Materials and Methods

Study Site

The study was conducted in the earthen ponds of the Aquaculture Unit located in the Agricultural Research and Experiment Station in Basra Governorate (College of Agriculture / University of Basra), which is located in Al-Hartha District, 16 km north of Basra (30^0 65' 64.6''N, 47^0 74' 79.5''E).

The station contains four earthen ponds with an area of 2500 m^2 and 14 earthen ponds with an area of 600 m^2. The current experiment was conducted in the large pond. The water was equipped with electric pumps (submersibles) from one branches of the Shatt Al-Arab river.

Fishes and Experimental Cages

Six cages made of polyethylene were manufactured for all treatments with dimensions of length × width × height (3 × 1.7 × 1.8 m) surrounded by external nets, the length of the mesh opening is 10 × 10 mm. These cages were placed inside the earthen pond after preparation, and the pond was filled with water at a height of 1.5m , and ventilation fans were placed to avoid the lack of oxygen during high temperatures.

A total of 600 common carp fish with an average weight of 163.41±10.16 g were transported from Babylon Governorate on 20 Feb 2020 by means of a transport vehicle designated for transporting fish equipped with water pumps for the purpose of supplying oxygen during the transportation process. The fish were transported Al-Hartha District / Agricultural Research and Experiment Station, and were placed in a one earthen pond for acclimatization for 10 days.

Fish were distributed in the cages at a density of 100 fish/cage. Two replications for each diet were used.

Feeding Management

Experimental diets were made in the feed production plant of the Agricultural Advisory Office of the College of Agriculture, University of Basra, where three diets were formulated, two diets with additives which included a control T1 (0% additives), T2 (1g/kg Thepax) and T3 (1g/kg

Endo Vit. C), the pellets size was 6 mm. Table (1) shows the ingredients used in the experimental diets, after calculating the average weight of the total fish in each cage, the daily food amount for each cage was calculated according to body weight and water temperature, and sampling repeated every 20 days to follow the growth and adjust the amount of food.

The experiment lasted for 233 days and fishes were fed six days a week, the daily feed quantity for each treatment was divided into three equal meals, the first feeding was in the morning (7 A.M.), the second was during the midday (12 P.M.) and the third was at afternoon (4 P.M.).

Table (1): Ingredients of the experimental diets (g/kg).

Ingredients	Control (T1)	Thepax (T2)	Endo Vit. C (T3)
Fish meal	250	250	250
Soybean meal	200	200	200
Wheat flour	310	310	310
Wheat bran	200	200	200
Vit. & mineral premix	20	20	20
Vegetable oil	20	20	20
Thepax	-	1	-
Vit. C	-	-	0.5

Environmental measurements

The most important environmental factors of water were measured during the study period at the days of sampling, all measurements were taken within nine A.M., and they included the following:

1) Water temperature was measured using a mercury thermometer divided from 0-100°C, where several readings were taken from the pond and then the average was calculated.

2) The pH was measured using a digital pH meter produced by the Italian company Hanna, by placing the sensitive part of it in the water, and the reading is recorded after its stability.

3) The dissolved oxygen in the water was measured by means of a digital electronic device “Oxygen meter” produced by the Romanian Martin company, by lowering the electrode

of the device into the water to different depths of 30-150 cm. After the stability of the reading, it is recorded and the average is taken.

4) Salinity was measured using a digital electronic device, EC meter, produced by the Italian company Hanna, by placing the sensitive part of it in water.

Growth performance

The experiment started at 4 March 2020, and at the end of experiment in 22 Oct.2020, the growth criteria were used to describe the growth performance of common carp:

Weight gain:

$WG = W_2\ (g) - W_1\ (g)$

Relative Growth Rate:

$RGR=[(W2\ (g) - W1\ (g))/W1] \times 100$

Daily Growth Rate (D.G.R.)

$D.G.R. = (W_2 – W_1) /(t_2-t_1)$

Condition Factor (K value)

$K\ value = (W / L^3) \times 100$

Specific Growth Rate:

$SGR= (\ln W_2\ (g) - \ln W_1\ (g) /(t_2-t_1) \times 100$

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.

Survival rate (%)

Survival rate = (No. of fish at the end / Total No. of fish) ×100

Feed utilization

Feed Conversion Ratio:

FCR= R (g)/ WG (g)

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

Protein Efficiency Ratio:

PER= WG (g)/ PI (g)

Protein Productive Value (PPV)

P.P.V. = (PG / PI) ×100

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

PG: weight of protein gain.
PI: weight of protein intake.

Chemical analysis

The experimental diets were analyzed according to A.O.A.C. (1990). Moisture was estimated by drying the samples at a temperature of 105 ^{0}C. The proteins were estimated using the Microkjeldahl device, and lipids was estimated using a Soxhlet apparatus**.** Ash was estimated by burning the samples in Muffle furnace at 550 °C for 4 hours. Fiber was determined gravimetrically after chemical digestion and solubilization of other materials present. The fiber residue weight is then corrected for ash content after ignition.

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

The feeding experiment data was analyzed with a completely randomized design, and the differences between the treatments means were tested by analysis of variance (ANOVA) and the significant differences were tested by LSD test at 0.05 probability level using SPSS program Ver. 22.

Results

Environmental measurements:

The values of environmental factors during the study period presented in Table 2, the highest water temperature was 30°C in July and the lowest was 21°C in March, while changes in pH were limited and its lowest value was 7.5 in March and the highest value was 8.1 in July, the concentration of dissolved oxygen was highest (7.2 mg / L) in March and the lowest concentration (6.5 mg / L) in July. Water salinity ranged from 3.13 psu in March to 4.84 psu in July.

Table (2): The environmental factors during study period.

Date	Temp. (°C)	pH	DO (mg/ L)	Salinity (psu)
4/3/2020	21	7.5	7.2	3.13
23/3	23	7.6	7.1	3.52
13/5	26	7.7	7.0	4.17

2/6	27	7.9	6.9	4.52
22/6	28	7.8	6.7	4.42
12/7	30	8.1	6.5	4.84
16/8	28	7.8	6.6	4.21
8/9	28	7.9	6.6	3.91
28/9	27	7.7	6.7	3.28
22/10/2020	24	7.6	7.1	3.61

Chemical analysis:

Table (3) shows the chemical composition of the experimental diet, where the percentage of crude protein was 27.84% and the moisture content was 7.51%. Table (4) indicates the body composition of common carp before and at the end of the experiment. It was noted in the table that the protein content of the Vit. C treatment was superior, which amounts to 17.94%, while it reached 16.10% for Thepax treatment, and the percentage of lipid was higher after the experiment than the value before the experiment in all treatments.

Proximate composition (%)	
Moisture	7.51±0.58
Crud protein	27.84±0.82
Crud lipid	6.39±2.47
Ash	9.86±0.54
Fiber	4.20
NFE	44.20±2.35
Gross energy (Kcal.kg^{-1})	4272.50±60.10

Table (3): Proximate composition of the experimental diet.

Table (4): Proximate composition of the common carp before and after experiment.

Proximate composition (%)	Before experiment	Treatments		
		Control	Thepax	Vit. C
Moisture	.7456 ±0.26	73.28 ±0.01	74.59 ±0.02	.7319 ±0.02
Crud protein	.1472 ±0.15	17.64 ±0.01	16.10 ±0.02	17.94 ±0.01
Crud lipid	.245 ±0.28	3.67 ±0.01	3.56 ±0.02	3.54 ±0.01
Ash	.766 ±0.22	4.82 ±0.01	5.17 ±0.02	4.72 ±0.02

Growth performance:

Table (5) shows the initial and final weight rates, and growth parameters of common carp for the different treatments. The highest value of the final weight was recorded in Thepax treatment (2209.34 g), followed by the Vit.C and the control treatment, which were 1792.70 g and 1631.62 g, respectively, and this is similar to the total weight increase, where the highest value in Thepax treatment (2035.65 g) and the lowest value was recorded in the control treatment (1478.23 g). As for the daily growth rate, the highest value was recorded in Thepax treatment, 9.83 g / day, followed by Vit.C and the control treatment. The results of the relative growth rate showed the superiority of Thepax treatment, followed by Vit. C and control treatments, which were 1174.74, 997.90 and 963.78% respectively. Also, Thepax treatment showed high specific growth rate, followed by Vit. C and control treatments, statistical analysis showed significant difference ($P \leq 0.05$) between Thepax treatment with both Vit. C and control treatments in all growth parameters, while no significant difference found between the last two treatments.

The survival rate was highest for Vit. C treatment, which reached 99.5%, then Thepax and control treatment recorded 99% and 83.5%, respectively.

Table (5):Growth performances of common carp in different treatments

(Mean± SD).

Parameters	Treatments		
	Control	Thepax	Vit. C.
IW (g)	153.39 ±0.22	173.70 ±11.09	163.14 ±3.85
FW (g)	1631.62 b ±82.38	2209.34 a ±9.21	1792.70 b ±180.88
WG (g)	1478.23 b ±82.60	2035.65 a ±20.31	1629.57 b ±177.03
DGR (g/day)	7.14 b ±0.40	9.83 a ±0.10	7.87 b ±0.86
RGR (%)	963.78 b ±55.23	1174.74 a ±86.73	997.90 b ±84.94
SGR (% /day$^{/}$)	1.14 b ±0.03	1.23 a ±0.03	1.16 b ±0.04
Survival rate (%)	83.50 b ±12.02	99.00 a ±0.00	99.50 a ±0.71

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

Feed conversion ratio (FCR), protein efficiency ratio (PER), productive protein value (PPV) and condition factor (K) were illustrated in Table (6). The results showed that the best food conversion ratio (3.14) for Thepax treatment, and showed significant difference (P≤0.05) with Vit. C and control treatment, the values of protein efficiency ratio showed similar trend where the highest value was in the Thepax treatment (1.215) and showed significant difference (P≤0.05) with control treatment (1.031), but Vit. C did not differ significantly with both other treatments. The treatments did not differed significantly in the value of productive protein value, where the third treatment, Vit. C, was superior over the other treatments and recorded the highest value (20.379%), and the lowest value in control treatment (18.495%). As for the condition factor, it reached the highest value (2.078) for Thepax treatment and the lowest value was for the control treatment, which was 1.897, also Vit. C treatment did not differ significantly with both other treatments.

Table (6): Feed utilization of common carp in different treatments (Mean± SD).

Parameters	Treatments		
	Control	Thepax	Vit. C.
FCR	3.69 b ±0.06	3.14 a ±0.25	3.42 ab ±0.25
PER	1.031 b ±0.018	1.215 a ±0.095	1.116 ab ±0.082

PPV%	18.495 a ±0.290	19.709 a ±1.514	20.379 a ±1.459
K	1.897 a ±0.037	2.078 a ±0.079	1.900 a ±0.067

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

Discussion

Water quality is the main factor for the success of fish farming (Björnsson & Ólafsdóttir, 2006), the water temperature ranged between 21 to 30 °C during the experiment period, i.e. from March to October, Hwang and Lin (2002) found that a high temperature of 35°C had a negative effect on the growth and FCR in common carp compared with 25°C, which was determined to be optimal, while Song-bo et al. (2012) found that maximum daily feeding rate reached at 28°C, which was in the range of present study.

The pH ranged between 7.5 - 8.1 during the experiment period, it was within the appropriate limits for common carp culture, according to FAO (2018) the appropriate pH for the culture of common carp ranges from 6.5 - 9.5. The salinity of the water in the present study where considered within the appropriate limits for common carp fish, although it is generally considered to be a stenohaline freshwater fish, but can tolerate a wide range of salinities (Salati et al. 2011). Dissolved oxygen concentration in all study period did not reached critical values below 3 or 4 mg/L (Bauer & Schlott, 2006).

The composition of fish body varies mainly with the type of food and water temperature (King, 2004). The results of the current study showed that the percentage of body protein was close in all treatments after the end of the experiment, but it was higher than the body protein before the experiment. Previous studies indicated that the protein content of common carp ranged between 14 to 18% (Cirkovic et al., 2010; Trbovic et al., 2009; Vladau et al., 2008), and these studies are consistent with the results of the current study. Also Al-Dubakel& & Al-Sanabani (2010) recorded the highest value for protein in the common carp fed diet containing 500 mg/kg vit.C.

The percentage of body lipid before the experiment was less than the percentage of lipid after the end of the experiment, Amancio et al. (2019) showed that an increase in the amount of food eaten by fish leads to an increase in lipid deposition in the muscles and viscera of the body as a result of consuming greater amounts of energy, this was noticed in the results of the current study, while body moisture before the experiment was higher than its percentage after the end of the experiment, According to many literatures, fish body lipid content are related closely to weight gain and inversely associated with moisture content (Barros et al., 2000; Yildirim et al., 2003).

The results obtained in our current study of growth indicators (final weight, weight gain, specific, relative growth rate and feed conversion ratio) when using Thepax compared to Vit. C and control treatments can be related to the stimulation of microorganisms in the gastrointestinal tract, which has a great role in increase in the surface area of the intestine, which leads to improve absorption of nutrients and thus leads to an increase in the efficiency of food utilization. (Azevedo et al., 2015; Anguiano et al., 2013). Although argument about

common carp requirement from vitamin C, Dabrowski et al. (1988) indicated the essentiality of this vitamin for this species, especially in early stages.

The results of the present study were consistent with the results of the study of Al-Jubouri & Saleh (2017) which found that Thepax treatment was significantly different from control, and Al-Mhanawi et al., (2021) that 1g/kg of Thepax has great potential as an important biomaterials to enhance the utilization of nutrients and metabolism by increasing the surface area of the intestines in young common carp fish, and it has a role in improving the digestive system, the villi length, villi width and villi area were significantly higher compared to the control diet. It was also similar to the study of Denji et al. (2015) when adding 1, 2.5, 4 g/kg of the prebiotic Mannan Oligosaccharide in the feeding of rainbow trout, which showed a significant superiority compared to the control diet in terms of final weight, specific, relative growth rate and feed conversion ratio. Kuhlwein et al. (2014) also obtained an increase in the values of weight indicators when adding (5, 10) g/kg fodder of the prebiotic beta-glucan to the feeding of mirror carp fish for eight weeks.

While the results of the present study did not agree with the study of Al-Dubakel et al., (2021) in grass carp (Ctenopharyngodon idella) when adding 1 g/kg of Thepax and 1 g/kg of feed of Vit. C, no significant differences were observed with the control treatment in growth indicators. Whereas the biological precursor consisting of MOS and Beta-glucan showed significant superiority compared to the control diet in terms of final body weight, specific and relative growth rate, weight gain and feed conversion ratio when adding 1.5 and 2.5 g/kg of feed to the diet of common carp (Eleraky et al., 2014). Also Tejpal et al. (2012) showed in a study that used live cells of S. cerevisiae and its extracts, that diets based on probiotics and prebiotics have a more positive effect on the growth of Clarius batrachus fish.

These result of food conversion rate agreed with what was indicated by Al-Mhanawi et al. (2021) that the rate of food conversion was increased over the rest of the treatments when 1g/kg of Thepax was added, while Yousefian et al. (2010) the best results in treatments received 0.15% and 0.2% from Aqualase (A commercial yeast probiotic composed of two yeast species S. cerevisiae and Saccharomyces elipsoedas, Nikkhoo et al., 2010) compared to 0.1% and control in the diet of common carp fingerlings.. Also the results of the current study showed a clear improvement in the utilization of protein in fish treated with Thepax, this was demonstrated by the values of the protein efficiency ratio (PER), while the control treatment gave the lowest value for the protein efficiency ratio, as indicated (Boostani et al., 2013) states that Thepax is a manipulated yeast cell supplemented with amino acids, this finding also similar with the results of Abdel-Tawwab et al.(2008) with S. cerevisiae as a growth supporter for Nile tilapia where the yeast supplementation significantly affected the whole fish body composition and suggested that yeast plays a role in enhancing feed intake (Banu et al., 2020).

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