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The Effect of Some Food Additives on Growth Parameters of Grass Carp *Ctenopharyngodon idella* Fingerlings

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Abstract: A laboratory experiment was carried out to investigate the effect of Thepax and Endo Vit. C as feed additives in the formulated diet on the growth performance of grass carp *Ctenopharyngodon idella*. The feed (30.11% crude protein) manufactured in the laboratory using raw local materials diet. Young grass carp (Average weight 3.36± 0.95 g) obtained from Aquaculture Unit ponds in Al-Hartha Station for Agricultural Researches, North Basrah. The fish were stocked in nine glass aquarium, three replicates for each treatment, of dimensions 60×40×30 cm provided with pumping aeration. The experimental diets included a control T1 (0% additives), T2 (1g Thepax/kg diet) and T3 (1g Endo Vit. C /kg diet) were used in feeding trail of current experiment. The experiment lasted for 57 days (from 29 Nov. to 24 Jan.) and fishes were fed six days a week using 5% of fish weight as feeding ratio. Results of current study reveals that grass carp fed on formulated feed supplemented with Endo vit. C having high growth performance (WG, DGR, RGR and SGR). The lowest Feed conversion ratio (2.511) was recorded in T3 this value was statistically significant (P≤0.05) with T2 while the highest value (2.767) was observed on the control. Protein efficiency ratio, feed intake and gross conversion efficiency were also recorded high values in T3, but control displayed superior values compared with Thepax treatment, which showed higher value of Relative feed intake compared to other two treatments.
Keywords: GCE, Thepax, Endo Vit. C, Grass carp.

Introduction

Feed represents about 50–80% of the production cost in aquaculture; therefore, suitable nutrition is one of the critical aspects to achieve a nutritionally balanced diet and low cost of production (El-Sayed, 2014; Hasan & Soto, 2017).

Feed additives were added to improve feed quality, health performance and feeding efficiency of the fishes. Most of these feed additives are non–nutritious and include antibiotics, immunostimulants, antioxidants probiotics, preservative, binder and colorants (Encarnação, 2016; Kord *et al*., 2021). Feed additives represent several types of molecules, compounds and even organisms that promote ingestion, absorption and assimilation of nutrients (Watts *et al*., 2020). Products that

improve feed efficiency are particularly important since feed costs are a major expense in aquaculture production (Nates, 2016).

Probiotics are live microorganisms which, when added in suitable amounts, confer a health benefit of the host (FAO/WHO 2002).Yeast was used as fed for farm animals for more than a hundred years (Owens and McCracken, 2007; Bajagai *et al*., 2016), it contains a high amount of enzymes, amino acids, fatty acids, vitamins and unknown growth factors, but in aquaculture only a few species were used, the most common was Saccharomyces cerevisiae (Encarnação *et al*., 2016; Agboola *et al*., 2021). Yeast (Thepax) acts as a probiotic in feed poultry (Yousefi & Karkoodi, 2007; Fazli *et al*., 2008; Zarei *et al*., 2011; Boostani *et al*., 2013) while other researchers consider Thepax as prebiotic (Nikpiran *et al*., 2013; Al-Mhanawi *et al*., 2021), however the manufactured companies cite it as prebiotic because its consist from cell wall of yeast Saccharomyces cerevisiae, mannan and glucan (Nikpiran *et al*., 2013). Most works about Thepax as food additive was applied in broiler performance nutrition such as Japanese Quails (Nikpiran *et al*., 2013) and broiler chickens (Boostani *et al*. 2013) which show positive effects on weight performance.

Vitamin C or ascorbic acid is an essential micronutrient for normal growth, antioxidant and immunity of fish, as they cannot synthesize this nutrient because of the lack of L-gulonolactone oxidase (Ching *et al*., 2015; Trichet *et al*., 2015; Adeyemi-Doro & Iyiola, 2018), therefore they depend on an exogenous source through the diet, moreover it is unstable and most of its activity in applied diets is lost during manufacturing and storage due to exposure to high temperature, oxygen and light. About 75% of the initial amount of supplemental vitamin C in feeds can be lost during processing at ambient temperature. However, appropriate requirements of vitamin C for grass carp *Ctenopharyngodon idella* juveniles remain unknown (Nasar *et al*., 2021).

Grass carp was the most widely cultured and commercially important freshwater fish species that consist about 11% of world aquaculture production (FAO, 2020), it is primarily an herbivorous fish that have a preference for aquatic vegetation (Zolfinejad *et al*., 2017). One of the main reasons for grass carp production increase related to the use of pelleted feed, that allow higher density or cage monoculture succeeded (Gan *et al*., 2012).

Little studies in Iraq on grass carp were achieved, and most studies deal with laboratory experiments (Al-Dubakel *et al*., 2011; Taher, 2017; Sayed-Lafi *et al*., 2018; Abdullah *et al*., 2020; Al-Dubakel *et al*., 2020). While few field studies were conducted (Al-Seyab, 1996; Saleh *et al*., 2008; Taher *et al*., 2021).

The objective of the current experiment was to assess the effect of Thepax and Endo Vit. C as feed additives on the growth performance of grass carp fingerlings.

Materials & Methods

A laboratory experiment was carried out to investigate the effect of Thepax and Endo Vit. C as feed additives in the formulated diet on the growth performance of grass carp. The feed manufactured in the laboratory using raw local materials (Table, 1) diet formulated to provide 27% crude protein. Grass carp (Average weight 3.36± 0.95 g) obtained from Aquaculture Unit ponds in Al-Hartha Station for Agricultural Researches, North Basrah. The fish after acclimation for seven days were stocked in nine glass aquaria of dimensions 60×40×30 cm

provided with pumping aeration, ten fish in three replicates for each treatment were used for the current experiment at the Laboratory of Live Food- Aquaculture Unit- Agriculture College. The experimental diets included a control T1 (0% additives), T2 (1g/kg Thepax) and T3 (1g/kg Endo Vit. C) were used in feeding trail of current experiment. The experiment lasted for 57 days and fishes were fed six days a week using 5% of fish weight as feeding ratio. All the fishes in each replicate were weighed every two weeks in order to adjust the feeding to new mean body weight.

Growth performance

The experiment started at 29 Dec.2020, and at the end of experiment in 24 Jan.2021 the following growth parameters were used to describe the growth performance of grass carp:

Weight gain:

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

Relative Growth Rate:

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

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.

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).

Feed intake:

Feed intake (FI) = 100 × total feed intake/[feeding days×$(W_1+W_2)/2$]

Relative Feed Intake:

$$RFI = FI/\ 0.5\times(W_2-W_1)\times d) \times 100$$

Gross Conversion Efficiency:

$$GCE(K) = SGR/\ RFI \times 100$$

Protein Efficiency Ratio:

$$PER = WG\ (g)/\ PI\ (g)$$

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

PI: weight of protein intake.

Chemical analysis Until the weight is stable

The artificial diets and alfalfa were analyzed according to A.O.A.C. (1990). The moisture content was estimated by drying the samples at a temperature of 105 ^{0}C until the weight is stable. 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

GE (Gross energy) was calculated according to NRC(1993) by using factors of 5.65, 9.45 and 4.22 Kcal per gram of protein, lipid and carbohydrate, respectively, and DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher *et al*. (1983).

Table (1): Ingredients of the experimental diet (g.kg^{-1}).

Feed Ingredients	Treatment (Diets)		
	T1	T2	T3
Wheat flour	300	300	300
Wheat bran	260	260	260
Fishmeal	200	200	200
Soybean meal	200	200	200
Vit. and minerals premix	20	20	20
Vegetable oil	20	20	20
Thepax[1]	0	1	0
Endo Vit. C[2]	0	0	1

[1]Thepax produced by Medro- Doxal, contains less than 10× 10^9 CUF/g of *Saccharomyces cerevisiae* var *Ellipsoideus*.

[2]Endo Vit. C produced by Medro- Doxal, contains 100% ascorbyl monophosphate.

Statistical analysis

The feeding trial was 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. 22.

Results

The proximate chemical composition of the experimental diet in table (2), shows that crude protein was 30.11%, the gross and digestible energy were 4372.205 and 3279.154 Kcal.Kg^{-1} respectively, while protein to energy ratio was 91.823 mg.Kcal^{-1}. Grass carp readily accepted all the experimental diets.

Table (2): Proximate chemical composition of the experimental diet (mean ±SD).

Proximate composition	(% Dry Matter)
Dry matter	92.46 ±0.03
Crude protein	30.11 ±0.11
Ether extract	6.84 ±0.06
Crude fiber	4.35 ±0.19
Ash	10.73 ±0.07
NFE	47.97 ±0.17
GE (Kcal/Kg diet^{-1})	4372.205 ±6.84
DE (Kcal/Kg diet^{-1})	3279.154 ±5.127
P/E ratio (mg.Kcal^{-1})	91.823 ±0.19

Present study reveals that grass carp fed on formulated feed supplemented with Endo vit. C having high growth performance (Table 3) i.e., WG, DGR, RGR and SGR values as compared to both control and feed supplemented with Thepax, these values are statistically highly significant (P≤0.05), meanwhile the later feeds best Feed conversion ratio (2.511) was recorded in fish fed on the diet supplemented with Endo vit. C followed by fish received Thepax , while the lower value (2.767) was observed on the control treatment. Protein efficiency ratio, Feed intake and Gross show no significant differences (P>0.05). In different periods of feeding trial, Thepax treatment exhibited higher values in average weight, but at the end and overall weight gain was greater in Endo vit. C treatment (Fig. 1).

After 57 days of feeding trial, there was significant variation in feed utilization of grass carp (P≤0.05), as indicated in table (4). The conversion efficiency were also recorded high values in Endo vit. C treatment, but control displayed superior values compared with Thepax treatment, which showed higher value of relative feed intake compared to other two treatments.

Table (3): Effects of various additives in feed on the growth performance of grass carp.

Growth parameters	Treatments		
	Thepax	Endo vit. C	Control
IW(g)	3.232 a ±0.128	3.203 a ±0.089	3.238 a ±0.051
FW(g)	5.068 b ±0.698	5.373 a ±0.873	5.118 a ±0.461
WG (g)	1.836 b ±0.798	2.171 a ±0.822	1.880 b ±0.411
DGR (g/day)	0.032 a ±0.014	0.038 a ±0.014	0.033 a ±0.007
RGR(%)	57.386 b ±26.868	67.561 a ±24.451	57.922 b ±11.688
SGR (%/day)	0.779 b ±0.289	0.894 a ±0.247	0.799 b ±0.127

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

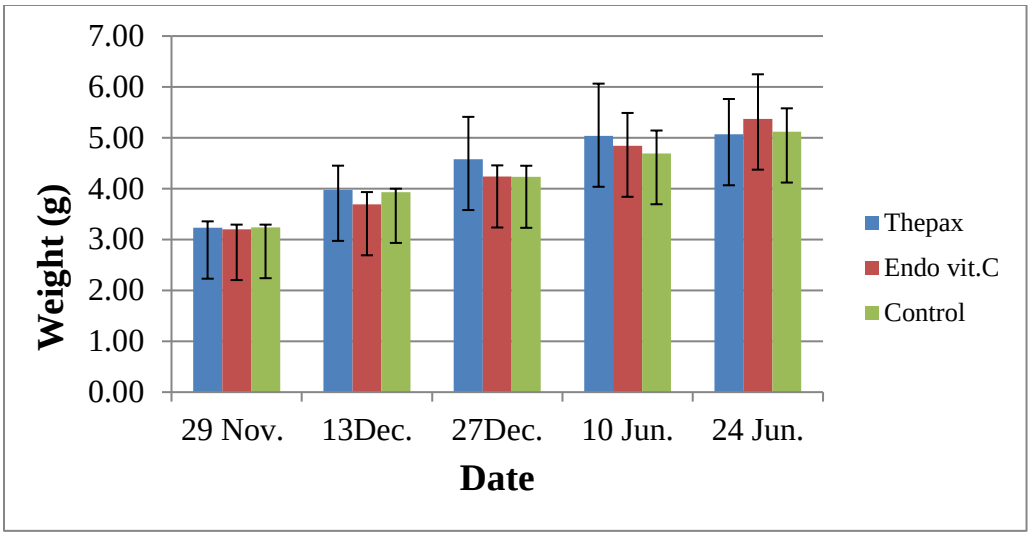

Fig. (1): Average weight of *Ctenopharyngodon idella* fed with different diets during the experimental period (Mean ± SD).

Table (4): Effects of various additives in feed on the feed utilization by grass carp.

Feed parameters	Treatments		
	Thepax	Endo vit. C	Control
FCR	2.688 b ±1.481	2.511a ±0.603	2.767 b ±0.406
FI	1.783 b ±0.619	2.098 a ±0.029	2.138 a ±0.017
PER	1.021 b ±0.092	1.497 a ±0.416	1.319 a ±0.211
RFI	4.100 a ±2.388	3.690 a ±1.184	4.111 a ±0.820
GCE(K)	11.338 b ±2.230	28.369 a ±19.382	20.502 a ±8.108

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

Discussion

In the present study, significant improvements in growth parameters which were (FW, WG, DGR, RGR and SGR) and feed utilization (FCR, FI, PER, RFI and GCE) indicates that supplementation of 1.0 g. kg Endo vit. C^{-1} promotes the growth performance of grass carp. The values of these parameters in the present study for Endo vit. C treatment was comparable with other studies, i.e., Lin & Shiau (2004) in grouper, *Epinephelus malabaricus*, Naggar & Lovell (1991) in Channel catfish (*Ictalurus punctatus*) and Al-Dubakel & Al-Sanabani (2010) in common carp. Li *et al*. (2018) studied grass carp and recorded higher values for growth parameters, but feed utilization indices were within the range of present results. Ascorbyl monophosphate which used in the present feed experiment seems to act more efficient as Vit. C source, it was chemically stabilized, generally by complexing Vit. C with phosphate to optimize its stability, therefore phosphorylated form of vitamin C is recommended (Wang *et al*., 2003), as a result it must therefore be supplied via the feed at recommended dosages for active uptake (Trichet *et al*., 2015; Omoniyi & Ovie, 2018; Luo *et al*., 2021).

The digestive physiology was affected by in-feed probiotics (Thepax) through improvement of intestinal enzyme activitiesa and significant increase in at least 11% in intestinal lactic acid bacteria population was observed (Adel *et al*., 2016). While vit. C acts as a reducing agent by being an electron donor to enzymes that assist in synthesizing collagen, carnitine, norepinephrine, peptide hormone, and tyrosine metabolism and have an important antioxidative role (Harrison & May, 2009; Adeyemi-Doro & Iyiola, 2018), however Thepax which used as food additive for grass carp in this study did not show significant differences with control and display lower values for both growth parameters and feed utilization compared with Vit. C treatment, this may be explained due to the small size of fish used in the present study and the alimentary canal was not completely developed so the benefits of the microbial flora balance of intestine did not achieved by Thepax (probiotic), while vit. C may have direct plays an important role in certain aspects of protein metabolism and has a specific effect on fish growth, also few studies about using this additive in fish diets showed that supplemented diet with Thepax enhance growth and immune responses in common carp *Cyprinus carpio*, but the values of FCR (7.71–7.93) were much higher than the present study (Al-Mhanawi *et al.*, 2021). Al-Jubouri & Saleh (2017) found that Thepax treatment was although significantly different from control but growth parameters of common carp values were less than other fourth treatments. Yousefian *et al*. (2012) also noticed that the differences of Aqualase (Thepax Aqua) treatment were not significantly with the control group for Kutum Fries (*Rutilus frisii kutum*). Also when local probiotic was used in the diets of young common carp, the inclusion of 4 and 6 $g.kg^{-1}$ show values approach to the control diet (Al-Dubakel *et al*., 2015).

Feed intake in Thepax supplemented diet showed high value compared with other two diets, this finding comparable with the results of Abdel-Tawwab *et al*. (2008) on *Saccharomyces cerevisiae* as a growth promoter for Nile tilapia, *Oreochromis niloticus* fry 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). The inclusion of Thepax in the present study was $1g.kg^{-1}$ which recommended by the manufacturer, it may be less than effective ratio as Adel *et al*. (2016) stated that inclusion rate 1.5% and above were significantly improved all growth performance parameters of rainbow trout *Oncorhynchus mykiss*. Nikkhoo *et al*. (2010) and Yousefian *et al*. (2010) got the best results in treatments received 0.15% and 0.2% compared to 0.1% and control in the diet of common carp fingerlings. While these rates are considerably higher than both the present study and manufacturer recommendation, this may be need further confirmation to use it in practical fish diets.

Gross conversion efficiency (GCE) related growth rate to feed intake of fish (de Silva & Anderson, 1995), while Bhilave *et al*. (2010) recorded very low values (0.0006 – 0.0014%) for GCE for *Labeo rohita*, Bhosale & Bhilave (2014) recorded high values (11.72 – 39.36%) for grass carp, both recent studies compared formulated feed with conventional feed for 90 days feeding trial. The results of present study were comparable for grass carp fed conventional and 100% replacement with

formulated feed in Bhosale & Bhilave (2014) study. Augustine *et al*. (2020) also recorded similar values for African catfish *Clarias gariepinus* fed varying levels of yellow maize.

Conclusion

The best growth performance and nutrient utilization recorded in grass carp fingerlings fed Endo vit. C as feeds additive, while Thepax did not show any benefits compared to control diet for small size of this species. A regular and adequate intake from exogenous sources for average growth is necessary and also to prevent vit. C deficiency.

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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.

Orchid

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تأثير بعض الاضافات الغذائية على معايير النمو لأصبعيات أسماك الكارب العشبي *Ctenopharyngodon idella*

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المستخلص: أجريت تجربة مختبرية في مختبر الغذاء الحي–وحدة الاستزراع المائي– كلية الزراعة لمعرفة تأثير كل من الثيباكس (Thepax) وفيتامين سي (Endo Vit. C) كإضافات غذائية في العلائق على أداء النمو لإصبعيات أسماك الكارب العشبي *Ctenopharyngodon idella* صُنعت العلائق (30.11٪ بروتين خام) مختبريا بأستعمال مصادر غذائية خام محلية. تم الحصول على أصبعيات أسماك الكارب العشبي (متوسط الوزن 3.36 ± 0.95 غم) من أحواض وحدة الاستزراع المائي في محطة الهارثة للبحوث والتجارب الزراعية التي تقع شمال مدينة البصرة. وضعت الأسماك في تسع أحواض زجاجية بواقع ثلاث مكررات لكل معاملة وبأبعاد60×40×30سم مزودة بمضخات التهوية. تضمنت العلائق التجريبية السيطرة (0% اضافةT1) والثيباكس 1غم/كغم (T2) و فيتامين سي 1غم/كغم (T3). استغرقت التجربة 57 يوماً، غذيت الأسماك ستة أيام في الأسبوع باستخدام 5% من وزن الأسماك كنسبة تغذية. أظهرت نتائج الدراسة أن أصبعيات أسماك الكارب العشبي المغذى على عليقة Endo vit. C اظهرت أداء نمو مرتفع في معايير (WG و DGR و RGR وSGR) وسجلت أقل نسبة تحويل غذائي (2.511) في T3 تليها T2 بينما أعلى قيمة (2.767) لوحظت في معاملة السيطرة. كما سجلت نسبة كفاءة البروتين وكمية الغذاء وكفاءة التحويل الكلية قيماً عالية فيT3، بينما أظهرت معاملة السيطرة قيماً أعلى مقارنةً بمعاملة Thepax، والتي أظهرت قيماً أعلى في معيار تناول الغذاء النسبي مقارنة بالمعاملتين الأخريين.

الكلمات المفتاحية: فيتامين سي، الثيباكس، كفاءة التحويل الكلية، الكارب العشبي.