ARTICLE / INVESTIGACIÓN

Assessment of Thepax and Bio Boost for promoting microbial growth in common carp intestines *Cyprinus carpio*

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DOI. 10.21931/RB/2022.07.04.18

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Abstract: The study aimed to evaluate the effect of Thepax and BioBoost as food additives on the microorganisms in the intestines of fish. From March 4 to October 22, the total number of common carp was 900 fish with an average weight of 163.41 ±10.16 g and a density of 100 fish/cage; three replicates were used for each treatment. The fish were fed three times daily. The included T1(0%additive), T2(1g/kgThepax)and T3 (1 g/kgBioBoost). The highest final weight value is Thepax treatment (2209.34 g), followed by Bio Boost and control. Microorganisms showed significant differences (P<0.05) in T2 for Lactobacillus sp. (10^2 x 65 CFU/ml) followed by T3 (10^2 x 55 CFU/ml) and control T1 (10^2 x 23 CFU/ml), also for Cellulomonas sp. in T2 (10^2 x 54 CFU/ml) followed by T3 (10^2 x 39 CFU/ml) and control T1 (10^2 x 7 CFU/ml). At the same time, *Aeromonas sp.* bacteria was higher in T1 (10^2 x 34 CFU/ml) over the treatments of T2 and T3 (10^2 x 2 CFU/ml) for both. We concluded the best additive was 1 g of Thepax / kg of feed in the recommended diets for common carp.

Key words: Thepax, Additives, Microorganisms, Intestines, Bacteria, Lactobacillus, Endo Bio Boost.

Introduction

Aquaculture is the fastest-growing food production and supports nearly 50% of all aquatic food for human consumption[1]. The main objective of aquaculture is to increase production[2]. During cultivation, fish may be exposed to various diseases, especially if they are stressed due to the deterioration of water quality and exposure to stressful conditions, which leads to significant economic losses[3].

Antibiotics in aquaculture lead to many severe problems, such as antibiotic-resistant bacterial strains and antibiotic residues in fish muscles, which would cause serious public health effects on human consumers and environmental pollution[4,5]. Therefore, finding safe and environmentally friendly alternatives is necessary to avoid the negative consequences of antibiotic use[6]. Most non-nutritive feed additives include antibiotics, immunostimulants, antioxidants, probiotics and prebiotics[7,8], which were added to improve diet quality, health, performance and feeding efficiency of fish[9]. Prebiotics are present in the cell wall of bacteria, yeasts and molds[10,11]. Prebiotics primarily affect the host by stimulating the selective growth of one or a limited number of non-pathogenic bacteria in the fish's gut, resulting in improved host health. Lactic acid bacteria are the most important, as they are part of the natural microorganisms of the fish gut. All their strains are non-pathogenic, in addition to their antagonism against many types of pathogenic organisms through their production of lactic acid[12,13]. The current study was conducted to evaluate the effect of Thepax and Bio Boost as feed additives on the microorganisms in the intestine of the common carp Cyprinus carpio.

Materials and methods

Study Site

The current study was accomplished in the ponds belonging to the Aquaculture Unit, which is located in the Agricultural Research and Experiment Station in Basrah Governorate (College of the Agriculture / University of Basrah), in Al-Hartha District, 16 km north of Basrah (300 65' 64.6"N, 470 74' 79.5"E). The current experiment was conducted in a large pond with 2500 m^2. The water was provided with electric pumps from one branch of the Shatt Al-Arab river.

Fishes and Experimental Cages

Experimental cages were made of polyethylene with dimensions of length × width × height (3 × 1.7 × 1.8 m) and enclosed by external nets (10 × 10 mm). The cages were placed inside the earthen pond which was filled with water at the height of 1.5m; ventilation fans were employed to avoid the shortage of oxygen, especially during high temperatures. There were 600 common carp fish with an average weight of 163.41±10.16 g distributed in the cages at 100 fish/cage density; two replications for each treatment were used.

Feeding Management

Experimental diets were produced at the feed production plant of the Agricultural Advisory Office, Faculty of Agriculture, University of Basra. Three diets were formulated, which included a control T1 (0% additive), T2 (1g/kg Thepax) and T3 (1g/kg BioBoost). The pellet size was 6 mm.

Citation: Al-Janabi M, Al-Noor J, Al-Dubakel A Y. Assessment of Thepax and Bio Boost for promoting microbial growth in common carp intestines Cyprinus carpio. Revis Bionatura 2022;7(4) 18. http://dx.doi.org/10.21931/RB/2022.07.04.18
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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The ingredients used in the experimental diets are presented in Table (1), and the daily amount of feed for each cage was calculated based on body weight. Sampling was repeated every 20 days to monitor growth and adjust the amount of diet. The experiment lasted 233 days (from March 4 to October 22). The fish were fed daily, and the daily amount of feed for each treatment was divided into three equal meals; the first was in the morning (7 A.M.), the second was during noon, and the third was in the afternoon (4 P.M.).

Environmental factors

Water's most significant environmental features were measured during the study period on the days of sampling. All measurements were taken within nine A.M., including water temperature, pH, salinity, and dissolved oxygen.

Growth performance

Growth criteria were used to describe the growth performance of common carp fed the experimental diets as follows:

Weight gain:

WG =Final weight (g) – Initial weight (g)

Survival rate (%):

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

Hematology

Blood was drawn from different groups of fish before and after the experiment. After cutting the caudal peduncle, the fish were held vertically with the head up to help blood flow by pressing on the caudal vein. Capillaries and tubes containing an anticoagulant were used. They were transferred to the laboratory to test the hematocrit, red blood cells (RBC) and white blood cells (WBC), according to Witeska *et al.*(2022).

Gut Sampling

Before and after the experiment, three fish were randomly sampled from each treatment and transported alive to the laboratory. The intestines were isolated from the body cavity using sterile scissors and forceps, and the contents were collected into sterile 2 ml tubes by squeezing with forceps.

Principle of the pour plate method

In this method, serial dilutions of the inoculum (serially diluting the primary specimen) are added within sterile Petri plates to which is poured melted and cooled (42-45°C) agar medium and completely mixed by revolving the plates, which are then left to solidify.

After incubation, the plates are observed for the appearance of individual colonies growing everywhere in the medium. The pure colonies of varying sizes, shapes and colors may be isolated/transported into test tube culture media to prepare pure cultures.

Tested bacteria

- *Lactobacillus spp.*
- *Falvobacterium spp.*
- *Aeromonas spp.*
- *Cellulomonas spp.*

Chemical analysis

The experimental diets were analyzed according to AOAC (1990), moisture, protein, lipid, ash and fiber were determined, while nitrogen-free extract was calculated according to New (1987) as follows:

%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 Statistical analysis

The experiment data were analyzed with a completely randomized design (CRD). The differences between the treatment 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. 26.

Results

Environmental factors

Table 2 shows the values of environmental factors during the study period, the highest water temperature in July

Ingredients	Control (T1)	Thepax (T2)	BioBosst (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	-
Bio Boost	-	-	1

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

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

Table 2. The environmental factors during the study period.

(30°C) and the lowest in March (21°C), while changes in pH were limited (7.5 -8.1), the concentration of dissolved oxygen ranged between 7.2 mg / l in March to 6.5 mg / l in July. Water salinity fluctuated from 3.13 PSU in March to 4.84 PSU in July.

Chemical analysis

The chemical composition of the experimental diet was presented in Table (3); the percentage of protein was 27.84% ±0.82, the lipid content was 6.39%±2.47, NFE was 44.20% ±2.35, and the Gross energy was 4272.50 Kcal.kg-1±60.10.

Table (4) shows the averages of initial and final weight and the total weight gain of common carp for the different treatments. The highest value of the final weight was recorded in the Thepax treatment (2209.34 g), followed by Bio Boost (1918.27 g) and the lowest in the control treatment (1631.62 g). This was similar for weight gain, where the highest value was in Thepax (2035.65g), and the lowest value was recorded in the Control treatment (1478.23g).

The blood parameters of common carp for different treatments showed in Table (5). It was noted that the hematocrit value did not differ significantly (P> 0.05) between the two treatments and the control. In contrast, all the hematocrit values for all the treatments were quite different (P<0.05) from the value of the pre-experiment fishes. Regarding the importance of red blood cells (RBC), it was significantly different (P<0.05) of the treatments of Thepax and BioBoost over the control treatment, which scored 1.543 and 1.533, respectively, then followed by the control treatments 1.257. While two treatments were significantly different (P<0.05) to the control treatment in the values of white blood cells (WBC) as Thepax and BioBoost treatments recorded 91.200 and 96.033, respectively, while the control treatment and pre-experiment recorded the least and amounted to 78.967 and 78.150 respectively.

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±0.36
NFE	44.20±2.35
Gross energy (Kcal.kg^{-1})	4272.50±60.10

Table 3. Proximate composition of the experimental diet.

Parameters	Treatments		
	Control	Thepax	Bio Boost
IW (g)	153.39 ±0.22	173.70 ±11.09	158.55 ±3.54
FW (g)	1631.62 b ±82.38	2209.34 a ±9.21	1918.27 b ±146.69
WG (g)	1478.23 b ±82.60	2035.65 a ±20.31	1759.73 b ±143.15

Table 4. Growth performances of common carp in different treatments.

Parameters	Before experiment	After experiment		
		Control	Thepax	BioBoost
HCT	8.300 b ±2.404	16.333 a 1.380 ±	16.733 a 2.250±	19.867 a 1.301±
RBC	1.365 b 0.177±	1.257 c 0.112±	1.543 a 0.177±	1.533 a 0.215±
WBC	78.150 b 9.546±	78.967 b 10.081±	91.200 a 7.702±	96.033 a 4.021±

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

Table 5. Growth performances of common carp in different treatments.

Gut microorganisms

Table (6) presented the numbers of bacteria in the intestines of common carp in different treatments. Where the number of colonies of *Lactobacillus* bacteria increased significantly (P<0.05) in the treatment of Thepax compared with the BioBoost treatment and control, as it recorded 10^2 x 65 cfu/ml and the BioBoost treatment recorded 10^2 x 55 cfu/ml followed by the control treatment which recorded 10^2 x 23 cfu/ml . In comparison, the highest rate of Aeromonas colonies was recorded in the intestines of fish in control, as it reached 10^2 x 34 CFU/ml. The colony numbers of these bacteria were significantly (P<0.05) reduced in the treatment of Thepax and BioBoost as it was recorded 10^2 x 2 CFU/ml for both treatments. The numbers of Flavobacterium increased significantly (P<0.05) in the control treatment, recording 10^2 x 33 CFU/ml. At the same time, it decreased in the Thepax and BioBoost therapy, as it recorded 10^2 X 3 CFU/ml and 10^2 X 7 CFU/ml, respectively. From Table (6), it was also noted that the number of colonies of cellulose-degrading bacteria Cellolomonas was significantly higher (P<0.05) in the Thepax treatment, recording 10^2 x 54 CFU/ml, followed by the BioBoost treatment with 10^2 X 39 CFU/ml, while the control treatment recorded 10^2 X 7 CFU/ml.

Discussion

Water quality was considered the main factor for the success of fish culture projects[14]; the water The temperature ranged from 21 to 30°C during the experiment, i.e., March to October. Hwang and Lin[15] found the optimum growth and FCR in common carp compared at 25°C. Song-bo *et al.*[16] observed the maximum daily feed intake at 28°C, which was in the range of the present study. The pH during the experimental period was within the appropriate limits for common carp culture, according to (16). The water salinity in the present study was within the applicable limits for common carp, which can tolerate a wide range of salinities[17,18]. Dissolved oxygen values in the entire study period were above 3 mg/l, and common carp optimal ranges between 4-8 mg/l[19,20].

The results obtained in the present study for growth parameters were in agreement with study[21], which found that the Thepax treatment was significantly higher than the control treatment. Al-Mhanawi *et al.*[21] found that 1 g/kg Thepax has great potential as an important component for improving nutrient utilization and metabolism by increasing the surface area of the intestine in common carp and has a role in improving the digestive system compared to the con-

Bacteria	Treatments	Mean (cfu/ml)	Std. Deviation (±)
Lactobacillus	Control	10^2 x 23 c	10^2 x 5.00
	Thepax	10^2 x 65 a	10^2 x 6.55
	Bio boost	10^2 x 55 ab	10^2 x 6.24
Aeromonas	Control	10^2 x 34 a	10^2 x 11.13
	Thepax	10^2 x 2 b	10^2 x 1.73
	Bio boost	10^2 x 2 b	10^2 x 1.00
Flavobacterium	Control	10^2 x 33 a	10^2 x 8.00
	Thepax	10^2 x 3 b	10^2 x 2.00
	Bio boost	10^2 x 7 b	10^2 x 2.00
Cellolomonas	Control	10^2 x 7 c	10^2 x 3.60
	Thepax	10^2 x 54 a	10^2 x 10.14
	Bio boost	10^2 x 39 b	10^2 x 6.55

Data in each row with different letters are significantly other ($P \leq 0.05$).

Table 6. Numbers of bacteria in the intestine of common carp in different treatments.

trol. The results were similar to those of study[22] by adding the prebiotic Mannan Oligosaccharide to trout feed, which showed a superior increase compared to other diets in terms of final weight. Al-Faragi[23] also increased weight indicator values by adding the prebiotic beta-glucan to common carp feed for eight weeks. While the results of the present study did not agree with those of Taher *et al.*[24] in grass carp (*Ctenopharyngodon Idella*) when adding 1 g/kg Thepax and 1 g/kg vitamin C to the diets, as no statistically significant differences were recorded with the control treatment in final weight and weight gain.

The survival rate of fish of the experimental therapies significantly increased compared to the control treatment. The best survival rate was recorded for the treatment of Thepax and BioBoost. A similar result was observed in the survival rate in the study of Tejpal *et al.*[25] on tilapia, where he confirmed that diets based on probiotics have a more positive effect on survival rate.

Blood parameters are considered one way to determine a fish's health in terms of stress and disease[26,27]. The present study's results agree with Akrami *et al.*[28], which revealed that common carp fed 1, 2, and 3 g/kg of the prebiotic MOS diet had significant superiority compared to the control in terms of hematocrit and white blood cells. The reason for this increase is due to the effect of the prebiotic on immune stimulation in fish. Modulation of the immune system is one of the generally proposed benefits of prebiotics and their ability to stimulate systemic immunity. Notably, several prebiotic supplements can even increase the rates of phagocytic cells, lysozyme cells and erythrocytes[27].

The results of the current study indicate a clear superiority of the additive treatments over the control treatment in the intestinal content of colonies of beneficial bacteria Lactobacillus and Cellolomonas, especially the treatment of Thepax. These results also indicate a decrease in the number of colonies of pathogenic and harmful bacteria Aeromonas and Flavobacterium for the additive treatments compared to the control treatment. The results of the current study agree with Ringo[28], in which probiotics were used in fish feed to enhance the immune response and increase fish resistance to diseases by increasing beneficial microorganisms in the intestine. Also, in the study of Yun Xia *et al.*[29] in which probiotics from Bacillus cereus and Bacillus subtilis were used as food additives for tilapia, the results showed a stimulating effect on the group of beneficial microorganisms in the intestine. After stopping the intake of probiotics by the fish for one week, the beneficial bacteria in the intestine were very few. As for the results of Lv *et al.*[30] study, it was in agreement with the results of the current research, as FOS was used, which reduced Aeromonas bacteria in the intestines of hybrid Nile tilapia. And the study of Dimitroglou *et al.*[1] where nutritional supplements (MOS) were used, as a result, gave a significant reduction in the number of harmful bacteria Aeromonas in the intestines of rainbow trout.

Conclusions

The addition of Thepax enhanced growth performance, feeding efficiency, survival rate, increased Lactobacillus count and low Aeromonas bacteria in the intestines of common carp.

Author Contributions

A short paragraph specifying their individual contributions must be provided for research articles with several authors. The following statements should be used "Conceptualization, Jalal M. Al-Noor. and Adel Y. Al-Dubakel.; methodology, Mohamed F. Al-Janabi.; software, Jalal M. Al-Noor.; validation, Mohamed F. Al-Janabi, Jalal M. Al-Noor. and Adel Y. Al-Dubakel.; formal analysis, Adel Y. Al-Dubakel; investigation, Mohamed F. Al-Janabi; resources, Jalal M. Al-Noor; data curation, Adel Y. Al-Dubakel.; writing—original draft preparation, Mohamed F. Al-Janabi.; writing—review and editing, Jalal M. Al-Noor.; visualization, Adel Y. Al-Dubakel.; supervision, Adel Y. Al-Dubakel.; project administration, Jalal M. Al-Noor; All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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