

INFLUENCE OF PHYTASE ENZYME ON GROWTH PERFORMANCE AND SURVIVAL RATE CHALLENGED WITH *SAPROLEGNIA SPP.* IN COMMON CARP

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ABSTRACT

This study was aimed to investigate the effect of phytase as feed additive on growth performance and survival rate against *Saprolegnia spp.* in common carp, *Cyprinus carpio* L. A total of 120 *C. carpio* (initial mass 20.05-20.35g) were randomly stocked into six treatments in duplicate (10 fish/tank) as follows: T1, T2 and T3 were fed basal diet supplemented with phytase at 1000, 2000 and 4000 IU/Kg diet respectively, while T4 were fed on basal diet plus β -glucan at concentration of 8.5 g/kg as well as the control group was fed basal diet without any addition of phytase. Results showed after 60 days of feeding period, that the addition of phytase enzyme was significantly ($p < 0.05$) affected on daily growth (DG), specific growth rate (SGR) and feed conversion ratio (FCR) in T3 and T2 followed by T4 and T1 compared to control group. At the end of experimental period (60 days) fish were challenged with *Saprolegnia spp.* After 15 days of challenge test, the best survival rate was recorded in T3 (100%) followed by T2 (80%) then T1 (70%) respectively. All treatment groups were better than the positive control group which recorded 40 %. Based on these results, this study approved the potency of the phytase enzyme on growth performance and survival rate against the Saprolegniasis in common carp.

Keywords: *Cyprinus carpio*- specific growth rate- water mold- phytase- feed conversion

فاضل ومصطفى

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تأثير إنزيم الفاييتيز على تحسين النمو ومعدل البقاء في اسماك الكارب الشائع المصابة بمرض عفن الماء

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المستخلص

هدفت الدراسة لمعرفة تأثير إنزيم phytase كأضافات علفية على علائق الاسماك في أداء النمو والمقاومة ضد الاصابة المستحدثة بالمرض الفطري عفن الماء *Saprolegniasis* في اسماك الكارب الشائع *Cyprinus carpio*. وزعت 120 سمكة (الوزن البدائي: 20.05 - 20.35) بشكل عشوائي في ستة معاملات وبمكررين (10 سمكة/معاملة) على النحو التالي: تم تغذية المعاملة الاولى والثانية والثالثة بنظام غذائي اساسي مع إنزيم الفاييتيز بمعدل 1000 و 2000 و 4000 وحدة دولية/كغم علف على التوالي في حين تم تغذية T4 على نظام غذائي اساسي بالإضافة إلى البيتاكلوكان بتركيز 8.5 غم/كغم بالإضافة الى مجموعة السيطرة بدون اضافة لانزيم الفاييتيز. أظهرت النتائج بعد 60 يوماً من فترة التغذية ، أن إضافة إنزيم الفاييتيز له تأثير معنوي ($P \leq 0.05$) على النمو اليومي (DG) ، ومعدل النمو النسبي (SGR) ونسبة التحويل الغذائي (FCR) في المعاملات الثالثة والثانية والرابعة والاولى على التوالي مقارنة مع مجموعة السيطرة. في نهاية الفترة التجريبية (60 يوماً) تم اختبار فحص التحدي في 10 اسماك من كل معاملة بفطر *Saprolegnia spp.* بعد 15 يوماً من اختبار التحدي ، تم تسجيل أفضل معدل للبقاء في المعاملة الثالثة (100%) يليها المعاملة الثانية (80%) والمعاملة الاولى (70%) على التوالي مقارنة مع مجموعة السيطرة الإيجابية التي سجلت 40%. بناءً على هذه النتائج برهنت هذه الدراسة الحالية على فاعلية إنزيم الفاييتيز على أداء النمو ومعدل البقاء ضد فطر السابرولكنيا في اسماك الكارب الشائع.

الكلمات المفتاحية: الكارب الشائع-عفن الماء-معدل النمو النسبي-إنزيم الفاييتيز- التحويل الغذائي

INTRODUCTION

Saprolegniasis in teleost fish is a major problem affecting both wild and farmed freshwater fish productions (15, 31). But, this disease is more abundant in the cultured fish which adversely affects fish industry (9). Several predisposing factors are responsible for increasing the susceptibility of Saprolegnia infections such as malnutrition, physical injury and bad water quality (23, 27). Infections of fish by these fungi generally cause lesions of cottony/woolly, white fungal growth over the skin, gills, or on fish eggs which subsequently become enlarge and could lead to death of fish (22). Recently, researchers have been suggested the use of feed additives to become an alternative way for the prevention and control of various diseases in aquaculture (16). Phytase used as feed additives in diet of common carp to improve growth performance; phytase enzyme increased nutrient absorption and regulated nutrient excretion, such as phosphorus, nitrogen, and minerals (5). Supplementation of phytase in fish feeds has been generally reported to improve the bio-availability and utilization of plant phosphorus by fish (3, 19). Microbial phytase is now be used as feed supplement. Many plant by-products have been successfully used in aquaculture without affecting the feed quality (3). Efforts are required to study the suitability of locally available plant by-products to enhance the fish production (8, 14). Some reported studies have been inconsistent as mentioned by Castillo and Gatlin, (4). Consequently, there is a required for further investigation to evaluate enzyme advantages in fish diet (14). Previous studies have not estimated the influence of phytase supplementation on immune response or resistance against disease and general health status of common carp. Hence, this study was planned to shed light on efficiency of the phytase on growth performance, and antifungal activity against *Saprolegnia spp.* in common carp.

MATERIALS AND METHODS

The current study was carried out in the Fish Diseases Laboratory at the College of Veterinary Medicine /University of Baghdad during the period extended from January 2019 until March 2019. A total of 120 of healthy *C.*

carpio (weight 20.05g – 20.35 g) used in this experiment which was obtained from a commercial farm (Al- Musayyib, Babylon). Initially the health status of the experimental fish was inspected, after that the fish were immersed in formalin at concentration of 37% (15ml/100L) for 30 min. or until the appearance of the stress on fish, after two weeks of acclimation for the fish before starting the experiment. During this time, they were stocked in two bath with dimension of 150 × 20 ×40 cm. Then, fish were randomly selected and distributed into 12 tanks at rate of 10 fish per tank (two replicates/treatment) were maintained for each of the six treatments (T1, T2, T3, T4 and control (positive and negative). Control was fed on basal diet only, T1, T2 and T3 were fed on basal diet + phytase enzyme at concentration of (1000, 2000, 4000 IU/Kg diet respectively), while T4 was fed on basal diet + β-glucan at concentration of 8.5g/kg diet. Fish were fed a rate 3% of body weight twice daily for 60 days. Every day tanks were cleaned and water was partially changed. Fish were weight every two weeks to determine growth performance according to the following equations:

1-Daily gain (D.G)

$$D.G = \frac{WT - Wt}{T - t}$$

WT = final body weight

Wt. = initial body weight; (T-t) = time

2-Specific growth rate (SGR %)

$$SGR = \frac{\ln W_2 - \ln W_1}{T} \times 100 \text{ (%g/day)}$$

SGR = percentage increase in weight per fish per day

Ln W1 = natural log of Initial weight of fish.

Ln W2 =natural log of final weight of fish.

T = Days of experiment (60 days).

3-Food Conversion Ratio (FCR)

$$FCR = \frac{\text{Total feed consumed by fish (g)}}{\text{Total weight gain by fish (g)}}$$

4-Food conversion efficiency (FCE)

$$FCE = \frac{\text{Total weight gain by fish (g)}}{\text{Total feed consumed by fish (g)}} \times 100$$

Preparation of diet

In this study, floating food was obtained from Faradanah Company (Iran) with a diameter of 5 ±0.4 was used as a basal diet. Diet was prepared by grinded the basal diet using food grinder and weighed individually feed for each treatment based of body weight. The procedure to prepare feeding experiment was first to dissolve an appropriate dose of phytase

enzyme into warm water (45°C) and then mix with basal feed (7). Then, different concentrations of phytase enzyme (1000, 2000, 4000 IU/kg diet) were added, these concentrations were selected based on previous study by Nwana and Schwarz (20) and 8.5g of β -glucan/kg and mixed well and converted into paste. These pastes were pelletized using food mixer with 1.5 mm diameter and dried at room temperature and air fun. The control group was fed with basal diet (commercial feed) without adding phytase. The food was prepared every week and stored in screw plastic container with moisture proof until feeding trial (30).

Isolation and identification of *Saprolegnia spp.*

Isolation of *Saprolegnia spp.* was carried out in the Fish Diseases Laboratory in the College of Veterinary Medicine/University of Baghdad. For the isolation of *Saprolegnia spp.*, water samples were collected from Tigris River of Baghdad/Iraq and Baiting technique was used for isolation of aquatic fungi (26). To obtain pure cultures from environmental, 15-20 ml of river water was poured into a sterile petri dish containing Chloramphenicol. Then, Sesame seeds, *Sesamum indicum* were added (5-7 seeds/ petri dish) (1), then incubated at 20 °C for 7 days and examined every day to observe the hyphae.

Identification of *Saprolegnia spp.*

Macroscopic appearance

The Macroscopic appearance included the color, shape, texture, and other characteristics features of colony of *Saprolegnia spp.* which observed on SDA and on sesame seed.

Microscopic examination

The identification of shape was performed by two ways:

Direct microscopic examination

This method included removal of infected area of samples and washed 2-3 times with sterile dH₂O and then transmitted to other clean slide and added 1-2 drop of Lactophenol cotton blue then was covered with cover slip and left for 2 min. Then, examined under high magnification (x40) to determine the shape of hyphae.

Indirect microscopic examination

The 2nd method was done by putting small part of fungal growth that cultured on SDA or part of sesame seed were taken on clean slide and

added 1-2 drops of lactophenol cotton blue stain, then covered with cover slip and left for 2 minutes and examined under light microscopy at high magnification (x40) to detect asexual organs (Zoosporangia).

Count the concentration of spore's suspension in one petri dish

The spore suspension was counted in 1 ml of solution using Newbaur hemocytometer chamber (10). Throw using pipits for WBC count with drawing the suspension to the degree of 0.5 and filled by PBS to the degree of 101 then mixed well for 3 min. and throw 3 drops of the suspension then putted one drop on slide chamber and by using the equation of WBC count, the concentration of suspension was adjusted to about 2×10^4 live spores /ml.

Challenge test

After 60 days of feeding trail, 10 fish in duplicate from each group were challenged with a viable fungal suspension (2×10^4 live zoospores/ml) of *Saprolegnia spp.* Control groups were divided into two subgroup: negative control (without challenge with *Saprolegnia spp.*) and positive control (fishes were challenged with a viable fungal suspension). Mortalities were recorded in all of the treatments for 2 weeks. Survival rate was calculated using the following formula: Survival rate% = final number of fish survivor/initial number of fish $\times 100$

Statistical analysis

Data were analyzed as mean and standard error (SE) using One-way analysis of variance (ANOVA) followed by Duncan's new Multiple Range Test (MRT) to compare the difference amongst the means. *P*-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Growth performance

The data on average body weight of *C. carpio* post dietary supplementation with phytase and β -glucan during 60 days are illustrated in Table 1. The body weight of all groups at one day of experimental period ranged between 20.05 -20.35 g and there were no significant differences observed in the initial weight among treatment group. In addition, similar trend was noted in the first 15 days of experimental period no significant differences among treatment groups. The mean final weight showed significant differences

($P < 0.05$) between T3 and other groups except T2. The improving effect of phytase was observed at 30, 45 and 60 days of experimental period, all groups showed significant increase ($P < 0.05$) among them. However, at 60 days of the experimental period, the growth performance exhibited significant increase ($P < 0.05$) for all treatment groups compared with the control group (28.20 ± 2.41). The highest weight gain (46.65 ± 3.72 g) was achieved in T3 followed by T2, T4 and T1 (43.95 ± 3.93 , 41.20 ± 3.72 and 35.50 ± 2.87 g, respectively). Moreover, fish fed on diet containing phytase and β -glucan had a significant ($P < 0.05$) increase in SGR(%) compared to fish fed basal control diet. However, there were no significant difference ($P \geq 0.05$) observed among treatment groups (T2, T3 and T4) in the SGR (1.37, 1.35% and 1.16) and DG (0.40, 0.43 and 0.34g/d) respectively. On the other hand, all treatment groups were showed significant differences ($P < 0.05$) in FCR compared with control group. But, no significant ($P \geq 0.05$) differences in FCR were observed among treatment groups (T2, T3, T4). Also, FCE was significantly improved by 4000 units/kg of phytase (i.e. T3) more than other 1000 and 2000 IU/Kg of phytase (T1 and T2). The highest value was recorded in T3 which was significantly increased ($P < 0.05$) in compared to T4, T2 and T1, respectively and to control group (Table 2). In the present study, phytase supplementation significantly ($P < 0.05$) increased the growth performance and FCR of *C. carpio* improved by supplementing the basal diet with phytase this was in agreement with results of other studies that have revealed the effects of phytase in increasing growth performance in fish. Rachmawati *et al.* (25) The addition of phytase enzyme in the artificial feed to Gift Tilapia Saline Fish, *Oreochromis niloticus* significantly increased the growth rate, protein efficiency ratio and phosphor digestibility, and decreased feed conversion ratio. Rachmawati and Samidjan, (24) showed that addition of phytase in feed significantly increased digestibility of feed, efficiency of feed utilization, and relative growth rate of common carp. The optimal doses of phytase based on ACDp, ACDf, EFU, RGR, PRR and FCR in common carp were

1040, 1100, 943, 988, 1000 and 1000 IU kg⁻¹ feed respectively. Also, Hussain *et al.* (13) found that phytase enzyme supplementation played a significant role in increasing nutrient digestibility and minimum amount of nutrients was discharged through faces at 750 and 1000 FTU kg⁻¹ phytase levels. Hussain *et al.* (12) reported that the maximum feed intake was also visualized at 750 FTU kg⁻¹ phytase level followed by phytase level of 1000 FTU kg⁻¹. The best FCR value (1.27) was also observed in the diet containing phytase level of 750 FTU kg⁻¹. The present study is in agreement with Xue, (32) who noticed that dietary phytase gave positive effect on feed intake, specific growth rate, and feed conversion ratio and daily gain of the Nile Tilapia. Also, phytase supplementation in the diets improved the apparent digestibility of Mg, total P, Mn and Zn in the fish body (11). As well Nwanna *et al.* (21) also reported significant increase in growth performance at 750 and 1000 FTU kg⁻¹ levels of phytase supplementation in diets of *C. carpio*. Further, Nwanna and Schwarz, (20) showed that the addition of 1000, 2000 and 4000 IU of phytase in the diets only marginally improved the growth performance. This is an indication, probably, that higher levels of phytase are required to promote significant growth differences and mineral absorption by the fish or perhaps the specific conditions in the gut of the stomach-less carp interfere with phytase. Results of this study agreement with Sardar *et al.* (28) indicated that dietary microbial phytase supplementation at 500 FTU kg⁻¹ diet without dietary DCP (dicalcium and phosphorus source), trace mineral premix and lysine and methionine supplementation could improve growth, weight gain, feed utilization in common carp. Liebert and Portz (17) concluded that the supplementation of phytase at 750 FTU kg⁻¹ is sufficient for maximum degradation of phytate in plant based diet resulting in higher growth performance of Nile tilapia, *Oreochromis niloticus*. Furthermore Baruah *et al.* (2) reported a significant increase in phosphorus digestibility due to dietary addition of citric acid (30 g/kg) in diets of Indian carp, *Labeo rohita* and this might be attributed to the growth improvement in fish to the beneficial effect of citric acid in releasing minerals from

the phytic acid complex of the soybean-meal-based diet. In like manner many studies have shown that the addition of phytase to diets has enhanced growth performance (3, 21). In the current study, the fed carp diets containing phytase showed significantly increased of the final weight, DG and SGR, FCE and FCR these results are in agreement with Debnath *et al.* (6) who showed that the phytase at 500 U/kg feed gave higher weight gain, apparent net protein utilization and energy retention value in *Pangasius pangasius* fingerlings. In the last, this indicated that the inclusion rate of phytase plays an important role in releasing phytate- phosphorus in plant protein-based fish diets and that the effectiveness of phytase varies with the plant ingredient source.

Survival rate

At the end of experimental period post challenge with *Saprolegnia spp.* the results showed differences concerning survival rate among treatments and control groups. The best survival rate values recorded in T3 and T4 which were 100% that mean no mortality followed by T2 which reached 80% then T1 which was 70% respectively. All treatment groups better than the positive control group which recorded 40 % survival rate (Table 3).

Survival rate results of the present study are in consistency with those of Shiao *et al.*(29) who found that nucleotide supplementation to the Nile tilapia's diet had a positive effect on the survival rate after being challenged by *S. iniae* compared to the non-supplemented group. In the present study the survival rate in positive control is in line with Mustafa *et al.* (18). For the phytase supplementation up to 4000 units/kg diet significantly improved the immune response and increased the survival rate of *C. carpio* fish after being challenged with *Saprolegnia spp.* In addition to Sardar *et al.* (28) who found that the supplementation of microbial phytase at 500 FTU kg⁻¹ diets of fish without supplemental dicalcium phosphates, trace mineral premix and lysine and methionine improved survival percentage of fish. While Yakupitiyage (33) found that the feed is not a factor that influences survival rate, because survival rate is affected by the initial treatment of the fish and quality of the cultivation media. Also, Yoo *et al.* (34) reported that survival rate was affected by fish gender, heredity, age, reproduction, disease resistance, and external factors such as water quality and density.

Table 1. Mean of body weight of *C. carpio* fed different levels of phytase and β -glucan during 60 days.

Treatment /Groups	Mean \pm SE				
	Zero day Weight (g)	15 days Weight (g)	30 days Weight (g)	45 days Weight (g)	60 days Weight (g)
Control	20.34 \pm 2.74 a	21.30 \pm 2.16 a	22.00 \pm 2.52 c	24.60 \pm 2.37 b	28.20 \pm 2.41 d
T 1	20.05 \pm 2.03 a	21.40 \pm 3.61 a	23.80 \pm 2.65 bc	28.20 \pm 3.09 b	35.50 \pm 2.87 c
T2	20.10 \pm 2.17 a	21.45 \pm 2.48 a	27.13 \pm 3.34 a	33.10 \pm 2.51 a	43.95 \pm 3.93 ab
T3	20.35 \pm 2.73 a	21.50 \pm 2.38 a	28.03 \pm 2.08 a	35.30 \pm 3.86 a	46.65 \pm 4.14 a
T4	20.35 \pm 1.86 a	21.65 \pm 3.07a	26.00 \pm 2.97 ab	32.10 \pm 2.92 a	41.20 \pm 3.72 b
LSD value	2.407 NS	2.191 NS	3.284 *	3.616 *	4.523 *

Mean values indicated by different letters within the same column are significantly different (P \leq 0.05)

Table 2. Growth performance (Daily gain, SGR%, FCR and FCE %) of *C. carpio* feeding different levels of phytase and β -glucan during 60 days.

Treatment Groups	Daily gain g/d fish	SGR (%)	FCR	FCE %
Control	0.13 \pm 0.05 b	0.54 \pm 0.04 c	4.71 \pm 0.09 c	21.21 \pm 0.71 d
T1	0.25 \pm 0.02 ab	0.95 \pm 0.09 b	2.53 \pm 0.11 b	39.38 \pm 1.37 c
T2	0.40 \pm 0.05 a	1.35 \pm 0.06 a	1.74 \pm 0.05 a	57.41 \pm 1.55 a
T3	0.43 \pm 0.06 a	1.37 \pm 0.06 a	1.68 \pm 0.05 a	59.27 \pm 1.92 a
T4	0.34 \pm 0.06 a	1.16 \pm 0.12 ab	2.02 \pm 0.04 a	49.32 \pm 2.62 b
LSD value	0.191 *	0.347 *	0.496 *	3.602 *

Mean with different alphabetic small letters in the same column are indicated significantly different, (P $<$ 0.05).

Table 3. Survival rate (%) of *C. carpio* treated with phytase and β -glucan follow up 15 days after challenged with *Saprolegnia* spp.

Treatment Groups		No. of fish	Follow up through 15 days		Survival rate (%)
			Mortality	Survive	
Control Negative		10	0	10	100
Control positive		10	6	4	40
T1	1000 UI/kg Phytase	10	3	7	70
T2	2000 UI/kg Phytase	10	2	8	80
T3	4000 UI/kg Phytase	10	0	10	100
T4	8.5 g/kg β -glucan	10	0	10	100

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