EFFICIENCY OF SYNBIOTIC AS FEED ADDITIVES ON GROWTH PERFORMANCE, SURVIVAL RATE AND HEALTH STATUS IN COMMON CARP CHALLENGED WITH *SAPROLEGNIA SPP.*

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ABSTRACT

The present study was conducted to investigate the effects of Synbiotic (combination of probiotic and prebiotic) as feed additive on growth performance, survival rate and immune response against *Saprolegnia spp.* in common carp. A total of 100 *C. carpio* fingerlings, weighing 49.55–50.50 g were randomly distributed into five treatment groups in duplicate, fishes were fed with different concentrations as follows: (T1) 0.5 %, (T2)1.0 % ,(T3) 1.5 % and (T4) 2% as well as the control group were fed basal diet without any addition of synbiotic. Results showed significant increase ($P \leq 0.05$) in all growth parameters (i.e. final weight, DWG, RGR, SGR (%), FCR, FCE) compared with the control group. The highest values were observed in fish fed on the diet containing 2% and 1.5% synbiotic. Also, survival rate recorded highest value (100%) in treatment supplemented with synbiotic 2% compared with control group (75%), suggesting that the high level (2%) improve growth rate and survival rate. At the end of experimental period six fish were randomly selected from each treatment and control (C) $^{+}$ for challenge test in a viable fungal suspension (1×10^5 live zoospores/ml) of *Saprolegnia spp.* Considerable changes have been observed in the mean values of WBCs. Respiratory burst activity showed significant increased in all synbiotic diet compared to C^{+} group. These results can be considered as a beneficial dietary for improving the growth rate and immune response in common carp.

Keywords: prebiotic-probiotics-respiratory burst activity-water mold

تأثير المرادف الحيوي كإضافة علفية في اداء النمو ومعدل البقاء وصحة أسماك الكار ب الشائع المصابة بمرض عفن الماء

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المستخلص

اجريت الدراسة الحالية لتقييم تأثير اضافة المرادف الحيوي (Synbiotic) والذي هو خليط من المعزز الحيوي (probiotic) والسابق الحيوي (prebiotic) كإضافات علفية الى علائق الاسماك في اداء النمو ومعدل البقاء والاستجابة المناعية في اسماك الكارب الشائع *Cyprinus carpio*. تضمنت التجربة استخدام 100 سمكة تراوحت اوزانها بين 50.50 – 49.55 غم وزعت عشوائيا الى خمس معاملات (بواقع مكررين لكل معاملة ولكل مكرر عشرة اسماك) اضافة الى مجموعة السيطرة (C) غذيت اسماك المعاملة الاولى (T1) على عليقة مضافا لها المرادف الحيوي (Synbiotic) بنسبة 0.5 % والثانية (T2) على عليقة مضافا لها المرادف الحيوي بنسبة 1% والثالثة (T3) بنسبة 1.5% اما المعاملة الرابعة (T4) بنسبة 2% في حين اعتبرت المعاملة الخامسة كمجموعة سيطرة (C)غذيت على عليقة بدون اي اضافة. بعد مرور 56 يوما من التجربة لوحظ زيادة في (الوزن النهائي ومعدل النمو اليومي ومعدل النمو النوعي و زيادة في كفاءة التغذية) في معاملة الاسماك (T4وT3) وتبعتها (T2وT1) والتي أظهرت زيادة معنوية مقارنة بمجموعة السيطرة. اجري فحص التحدي للمرض الفطري($1x10^5$ سبورات فطرية/مل) في نهاية التجربة بواقع 6 سمكات لكل من المعاملات الاربعة اما مجموعة السيطرة (C) فقد قسمت جزئيين C^{-}وC+ . سجل اعلى معدل للبقاء في معاملة T3 مقارنة بالسيطرة (75%) بعد مرور 12 يوم من اجراء فحص التحدي تم اجراء الفحوصات المناعية والتي اوضحت النتائج ان المعاملتين T4وT3 تفوقتها معنويا ($P<0.05$) مقارنة بمعاملة السيطرة والمعاملتين T2وT1 في عدد كريات الدم البيضاء والتي اظهرت نسبة خلايا اللمفوسايت والفعالية البلعمية للنيتروفيل زيادة معنوية في المعاملات T3و T4 مقارنة بمجاميع السيطرة الموجبة والسالبة كذلك اظهرت فعالية الانشطار التنفسي زيادة معنوية في جميع المعاملات المغذات على المرادف الحيوي مقارنة بمعاملة السيطرة (C^{+} , C^{-}) .

الكلمات المفتاحية: المعزز الحيوي، المرادف الحيوي، فعالية الانشطار التنفسي

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INTRODUCTION

Improvement and protection of fish health in commercial production practices is a major factor in the aquaculture industry. A novel approach to these goals is application of probiotics and prebiotics in fish farming industry (33). The global demand for safe food has provoked the search for natural and alternative growth promoters to use in fish feeds (32). Antibiotics used as traditional strategy for fish diseases management but also for the improvement of growth and efficiency of feed conversion, however, the development of antimicrobial resistant pathogens were recognized but there is a huge risk of transmission of resistance bacteria from aquatic environment to human (20). Probiotics are defined as microbial dietary adjuvant that beneficially physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (15). Probiotics have been proven to play positive roles as feed additives in various aspects such as growth performance and disease prevention. While, Prebiotics are non-digestible food ingredient that stimulate the growth or activity of beneficial gut commensal bacteria in host thus improves host health (14). The optimum dose of probiotic is depending on the host and the immune parameters expected to be induced; therefore, the dose of each probiotic must be determined for each host species (19).The combination of probiotics and prebiotics in so-called synbiotic, synergistic effects can be achieved (21)**.** Such a combination could improve the survival of the probiotic organism because without its food supply, a probiotic does not survive well in digestive systems as it cannot tolerate oxygen, low pH and temperature conditions (8). The combined effect of prebiotics and probiotics application is greater benefits than the application of individual probiont (17). The use of probiotics and prebiotics has been well considered as an alternative viable therapy in fish culture. This strategy offers innumerable advantages to overcome the limitation and side effects of antibiotics and other drugs and also leads to high production (23). A large body of literature indicates that dietary use of probiotics, prebiotics and synbiotic effectively enhances growth performance in aquatic and other animals (4). These alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immune responses and improve growth performance (26).Therefore, the current study aimed to investigate the efficiency of dietary synbiotic on growth performance**,** survival rate and immune responseof common carp.

MATRIALS AND METHODS

Fish and experimental condition

A total of 100 of healthy fingerlings of *C. carpio* weight ranged between 49.55 - 50.50 g used in this experiment which was obtained from a commercial hatchery (Al–Mahaweel, Babylon). The present study was carried out at College of Veterinary Medicine/ University of Baghdad, Ichthyology laboratory, Baghdad, Iraq. Initially the fish were immersed in a salt bath of NaCl at conc. of 3% to get rid of external parasites and fungal infections. After that, two weeks of acclimation for the fish before starting the experiment. During this time, they were stocked in two bath trough with dimensions of 150 × 20 ×40 cm. Then, 100 fish were randomly selected and distributed into 10 tanks trough at rate of 10 fish per tank (two replicates /treatment) were maintained for each of the five treatments (T1, T2, T3, T4 and control) Tanks with dimensions of 40 × 70×70 cm were filled with 80 L chlorine–free tap water. Chemo-physical parameters of the water were recorded daily during the experimental period such as water temperature ranged between 24-26ºC, pH of water ranged between 7.2-7.6 and the concentration of dissolved oxygen (DO) ranged between 6.5- 7 mg/I. These values are considered suitable limits for fish living and growth of common carp, (11). Fish, were fed a rate 2% of body weight twice daily for 56 days (3). Fish were weighted every 2 weeks to determine different growth parameters. Pre and post challenge test, blood samples were collected to evaluate immunological parameters (WBCs, and (NBT). Also, post challenge test (in a viable fungal suspension at conc. of 1×10^5 live zoospores/ml) the mortality rate was recorded.

Experimental Diets

Floating food 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 on 2% of body weight. Different level of synbiotic used in this trail was consist of (local probiotic 0.5%) [(*Saccharomyces serevisiae* 10^{10} CFU/ml, *Bacillus subtilus* 10^{9} CFU/ml and Lactic acid bacteria J6 10^{11} CFU/ml)] and ß-glucan (1%) as the prebiotic. A basal diet was formulated (protein 36%, carbohydrates 29%, fat 9%, ash 10%, fiber 5%, moisture 10% and Phosphorus 1.1%) this basal diet served as the control diet without adding synbiotic. Different concentrations of synbiotic was added to basal diet (0.5%, 1%,1.5% and 2%). and mixed well and converted into paste. These pastes were pelletized using food mixer with 1.5- mm diameter and dried at room temperature. The diet was prepared every week and stored in screw plastic container at 4°C until feeding trial (27).

Growth performance: Growth weight was calculated every two weeks

Body weight gain: It was estimated according to Schmalhusen (24). Final fish weight(g) –initial fish weight (g).

Daily gain (D.G): It was calculated according to Sahu et al. (23). D.G = $\frac{WT-Wt}{T-t}$

Specific growth rate (SGR%): It was calculate according to the following equation to (7). Brown

$$SGR=\frac{(\ln W2-\ln W1)}{T}\times 100 \ldots\ldots \text{(\%g/day).}$$

Feed Conversion Ratio (FCR): It was estimated by the following equation according to Uten (31).

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

Feed conversion efficiency (FCE): It was estimated by the following equation according to Uten (31).

$$FCE=\frac{\text{Total weight gain of fish (g)}}{\text{Total food intake by fish (g)}}\times 100$$

Survival rate

The fish were counted at the end of experimental period (at 56 days from the beginning of experiment) to determine the survival percentage, according to Amend (5).

Survival%= No. of fish counted at 56 days/ No. of stocked fish x 100

Immunological parameters

Total White Blood Cells (WBCs) Count

WBCs count was performed as described by Thrall et al. (30).

Total WBCs count = N/4× 20×1/10 Cell/mm^3

Respiratory burst activity

Reactive oxygen radical production by neutrophils during respiratory burst activity was evaluated by the reduction of nitro bluetetrazolium (NBT) to Formosan. Blood samples were mixed with 0.2% NBT in equal proportion (1:1) and incubated for 30 min at 25ºC. 50µl of this mixture was taken out and 1 ml of dimethyl formamide (SRL, India) was added to solubilize the reduced Formosan product. Then it centrifuged at 2000 rpm for 5 min and the supernatant were taken. The reduced extent of NBT can be measured at an optical density of 540 nm with dimethyl formamide as the blank using Spectrophotometer according to methods described by Siwickiet et al. (25).

Statistical analysis

All values were statically analysis using SAS (Statistical Analysis System –version 9.1). One –way analysis of variance (ANOVA) with Least significant differences (LSD). $P\leq 0.05$ was considered significant difference

RESULTS AND DISCUSSION

Growth performance

The growth performance of *C. carpio* post treatment with different levels of dietary synbiotics during 56 days is presented in Table 1. The body weight of all groups at one day of experimental period ranged from 49.55 to 50.50 g and there were no significant differences observed in the initial weight among them and control treatment. Also, similar trend was noted in the first 14 days no significant differences among treatment groups. The mean final weight showed no significant differences ($P>0.05$) between T3 and T4 (79.65, 84.20 g, respectively). The improving effect of synbiotic was observed at 28, 42 and 56 days because all groups showed significant increase ($P \leq 0.05$) among them. However, at 56 days, the growth performance exhibited significant increase ($P\leq 0.05$) for all treatment groups accept T1 compared with the control group which was 65.71g. The highest weight gain (84.20 g) was achieved in T4 followed by T3, T2 and T1 (79.65, 73.95 and 68.31g, respectively)

Table 1. Average body weight of *C.carpio* fed in different concentrations of synbiotic during 56 days

Treatment	Mean ± SE				
	Zero day	14 days	28 days	42 days	56 days
	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Weight (g)
Control	50.05 ± 2.74 a	51.45 ± 2.16 a	53.6 ±2.52 b	60.71 ± 2.37 b	65.71 ± 2.41 b
T1	49.55 ± 2.03 a	53.55 ± 3.61 a	59.45 ± 2.65 a	63.35 ± 3.09 b	68.31 ± 2.87 b
T2	50.45 ± 2.17 a	53.55 ± 2.48 a	61.42± 3.34 a	66.65 ± 2.51 ab	73.95 ± 3.93 ab
T3	50.45 ± 2.73 a	56.65 ± 2.38 a	62.94 ± 2.08 a	70.65 ± 3.86 ab	79.65 ± 4.14 a
T4	50.50 ± 1.86 a	54.30 ± 3.07 a	63.66 ± 2.97 a	72.5 ± 2.92 a	84.20 ±3.72 a
Level of Sig.	NS	NS	*	*	*

Means Values indicated by different letters within the column are significantly different * (P<0.05)

FCE% was significantly difference (P≤0.05) in groups treated with synbiotic accepted in T1 group (27.8) compared with control group (23.2). Also, there were no significant difference (P>0.05) observed in the BWG (29.20, 33.70g, SGR(0.81, 0.91%) and DG (0.33, 0.41g/d) between treatment groups T3 and T4. On the other hand all treatment groups were showed significant differences (P<0.05) in FCR (3.5, 2.8, 2.30 and 1.99) compared with control group (4.3). The highest value was recorded in T4 which was significantly increased (P<0.05) compared to T3, T2 and T1, respectively and to control group. The obtained results could be attributed to the selective modification of intestinal microbiota through synbiotic (9). Consequently, improving digestibility and growth performance. Probiotics have been shown to produce digestive enzymes such as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes. Similar data obtained by (12]. noticed that dietary synbiotic Biomin IMBO (*Enterococcus faecium* and Fructooligosaccharide) (FOS) at three levels 0.5, 1.0 and 1.5 g kg^{1} for 60 days significantly increase the final mean weights and specific growth rates (SGR), survival rate and reduced FCR in rainbow trout, fingerlings. In the present experiment, the growth performance, were significantly (P<0.05) improved by supplementing the basal diet with synbiotic (16). assessed that dietary inclusion of a commercial synbiotic, Biomin IMBO (Biomin, Herzogenburg, Austria), containing different levels of synbiotic (0.5, 1.0 and 1.5), comprised of a probiotic (*E. faecium* 5 $\times 10^{11}$CFU/kg) and FOS as a prebiotic, in rainbow trout fed for 60 days gave a rise to a body weight gain of about 50, 59 and 53%, respectively, in comparison to the control group. In contrary, (1) reported that no significant interactions were observed between dietary *Bacillus subtilis* and fructooligosaccharide (FOS) on the specific growth rate and survival rate of large yellow croaker. The experimental fish was healthy and no mortality was observed during the feeding trial. Japanese flounder feeding *B. clausii* and MOS/FOS, in which fish maintained active ingestion, exhibited proper growth and survived for all time (34). While, the growth parameters, almost all references reported a positive effect of synbiotics on any parameter (22).The lowest survival rate in C+ group are in line with (18) who showed higher mortality rated in C+ group following challenge with Saprolegnia. Also, these results are in agreement with (10).

Table 2. Growth performance (body weight gain, daily gain, SGR%, FCR and FCE %) of *C. carpio* feeding different concentrations of synbiotic after 56 days

Treatment	Parameters				
	Body weight gain (g)	Daily gain g/d fish	SGR (%)	FCR	FCE %
Control	15.35 ± 1.02 c	0.27± 0.05 b	0.48± 0.04 b	4.3 ± 0.09 d	23.2 ± 0.71 d
T1	18.76 ± 1.25 bc	0.33± 0.02 ab	0.56± 0.09 b	3.5± 0.11 c	27.8 ± 1.37 d
T2	23.50 ± 1.09 b	0.41± 0.05 ab	0.68± 0.06 ab	2. 8± 0.05 b	34.89± 1.55 c
T3	29.20 ± 2.16 a	0.52± 0.06 ab	0.81± 0.06 a	2.30± 0.05 b	43.3 ± 1.92 b
T4	33.70 ± 1.52 a	0.60± 0.06 a	0.91± 0.12 a	1.99± 0.04 a	50.04 ± 2.62 a
Level of Sig.	**	*	*	**	**

Means values indicated by different letters within the same column are significantly different *(P≤0.05)

Survival rate

At the end of experimental period (56 days) all groups T1, T2, T3 and T4 received synbiotic diets revealed significant decrease in mortality rate when compared with control groups. The best survival rate values were showed in T4 which reported 100% that mean no mortality than T3 and T2 which reached 95% followed by T1 which 85% respectively. All treatments seemed better than the control group which recorded 75 % survival rate and 25% mortality rate (Table 3).

Table 3. Survival rate of *C. carpio* treated with synbiotic during 56 days.

Treatment	Total number	Number dead fish	Mortalities%	Survival rate%
Control	20	5	25	75
T1	20	3	15	85
T2	20	1	5	95
T3	20	1	5	95
T4	20	zero	zero	100

Immunological parameters

Total white blood cells (WBCs) count

Data of WBC count pre and post challenge with *Saprolegnia spp.* is summarized in Table 4. Fish treated with synbiotic supplemented diets (T1, T2, T3 and T4) showed significant increase (P≤0.05) in WBC count pre challenge than those of control group. The highest values were recorded in T4 followed by T3 which was (37.9× 10^3/mm3 and 36.1 × 10^3/mm^3) and there was no significant difference (P>0.05) between T3 and T4. Similarly there was no significant difference (P≤0.05) between T1 and T2 (29.3 × 10^3/mm^3 and 31.5× 10^3/mm^3). However, in all treatment groups the WBCs were significantly increased (P< 0.05) compared to the control group (C-, C+) which was (22.8× 10^3/mm^3 and 24.82 × 10^3/mm^3). On the other hand, WBCs count post challenge showed significant increase (P< 0.05) in fish treated with synbiotic supplemented diets than the pre challenge. The highest values were recorded in T4 (39.2 ×10^3 cell/mm3) followed by T3,T2 and T1 (37.2 × 10^3/mm^3, 33.2× 10^3/mm^3, 32.2 ×10^3cell /mm^3) compared to the control (C+)group (27. 47 ×10^3 cell /mm^3). Also there was a significant difference between (C-) group which was 23.5×10^3 cell /mm^3 than the (C+) group (27.47× 10^3 cell/ mm^3) in post challenge. All treated groups with synbiotic supplemented diets showed no significant differences (P>0.05) in WBC count between pre challenge and post challenge. The WBCs are one of the most important cells that can stimulate immune responses of fish and serves as one of the first line of body defense and their numbers increase sharply when infections is established arise (20). The results of total leukocyte count of *C. carpio* groups treated with synbiotic/ probiotic and β- glucan

as prebiotic showed significant increase of WBCs count compared with control groups. These results were fully accordant with those reported by (29) who noticed that snakehead, *Channa striata* fingerlings feed with supplemented diets *(L. acidophilus* and yeast β-glucan) the number of WBC was significantly increased in all post-challenged groups than the pre-challenged groups and control groups. On contrary, (34) reported that dietary of MOS had no effect on hematological parameters (WBC) of Channel catfish, *Ictalurus punctatus*. The current results indicated that stimulation of non-specific immune response in *C.carpio* group due to their feeding additive diet with synbiotic (probiotic and prebiotic) (β-glucan).They were manifested by increased number of WBCs by the released factors that stimulate hematopoietic organs to produce more leukocytes (28). As well as, the effect of β –glucan (yeast cell wall structures mainly; β–glucan) which has specific receptors on the phagocyte cells, (neutrophils and monocytes) (36).

Table 4. WBCs count of *C. carpio* fed different concentrations of synbiotic diet pre challenge and post challenge by *Saprolegnia spp.*

Treatment	Mean ± SE of WBC (10^3/mm^3)		T-Test
	Pré challenge	Post challenge	
Control (C-)	22.8 ± 1.73 c	23.5 ± 1.07 e	-
Control (C+)	24.82 ± 1.06 c	27.47 ± 1.33 de	
T1	29.3 ± 2.07 b	32.2 ± 2.42 cd	3.27 NS
T2	31.5 ± 2.66 b	33.2 ± 1.94 bc	3.09 NS
T3	36.1 ± 3.06 a	37.2 ± 2.35 ab	2.71 NS
T4	37.9 ± 2.94 a	39.2 ± 3.17 a	2.50 NS
Level of Sig.	4.841 *	5.163 *	---

Means values indicated with different letters the in same column are significantly different (P<0.05).*

Respiratory burst activity

The result of respiratory burst activity (NBT reduction) of *C.carpio* neutrophils of the experimental groups pre and post challenge with *Saprolegnia spp.* were presented in Table 5.The production of superoxide examined by NBT reduction was significantly influenced by dietary supplementation of synbiotic which indicated by significant increase (P≤0.05) in all treatment groups compared with control group. After 56 days of feeding trail, optical density of NBT showed significant difference (P≤0.05) between T1 and T2 (1.15, 1.38 respectively). However, there was no significant difference between T3 and T4 (1.45, 1.43) compared to control group (0.51). Post challenge with *Saprolegnia spp.* respiratory burst activity increased significantly (P<0.05) in all synbiotic supplemented groups compared to control group (C-) and (C+) which was 0.52 and 1.00. T4 showed the highest level of NBT reduction which was significantly different over the other groups and control that were 1.52, 1.49, 1.40, and1.19 compared with control (1.00), respectively. The ability of macrophages to kill pathogenic microbes is probably one of the most important mechanisms of protection against diseases among fish. The free oxygen radicals and nitric oxide are the most destructive products produced by activated macrophages. The present study showed significant enhancement of the neutrophils/macrophages activity than in control group. Increase in respiratory burst activity was observed in all groups which were in agreement with previous finding of (13). who found that after 8 weeks of feeding synbiotic combination, *Pediococcus acidilactici* and galactooligosaccharides of rainbow trout significantly enhanced activities of respiratory burst indication of the

potential of reactive oxygen species. Increase in observed in all groups which were in agreement with previous records of (6). In contrast the respiratory burst of head kidney leukocytes was not affected by dietary administration of FOS and *B. subtilis* of yellow croaker (1).

Table 5. Respiratory burst activity (NBT reduction) of *C.carpio* neutrophils pre challenge (after 56 days feeding) and post challenge by *Saprolegnia spp.*

Treatments	Mean ± SE of NBT	
	Pre challenge	Post challenge
Control (-Ve)	0.51±0.02 c	0.52± 0.04 c
Control (+Ve)	0.54 ± 0.03 c	1.00 ± 0.14 bc
T1	1.15 ± 0.05 b	1.19 ± 0.05 b
T2	1.38 ± 0.11 a	1.40 ± 0.10 a
T3	1.45 ± 0.06 a	1.49 ± 0.07 a
T4	1.43 ± 0.05 a	1.52 ± 0.09 a
Level of sig.	*	*

Means values indicated with different alphabetic letters in the same column are significantly different (P<0.05)*

REFERENCES

1. Ai, Q. H. Xu, K. Mai, W. Xu, J. Wang and W. Zhang. 2011. Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture, 311: 155-161

2. Akrami, R., M. Nasri-Tajan, A. Jahedi, M. Jahedi, M.R. Mansour and S.A. Jafarpour. 2015. Effects of dietary synbiotic count, blood indices and immunity of beluga (*Huso huso* Linnaeus, 1754) juvenile. Aquacult. Nutr. 21: 952-959

3. Akrami, R. 2010. The effects of mannan on growth, survival, lactobacillus bacterial oligosaccharide on growth, survival, body composition and resistance of Caspian Sea white fish (*Rutilus rutilus*). J. Mar.Sci. and Technology, 21: 8-14

4. Al-Faragi, J.K. and S.A.A. Al- Saphar, 2013. Effect of local probiotic on common carp *Cyprinus carpio* growth performance and survival rate, J. Genet. and Envir. Res. Conservation, 1: 89-96

5. Amend, D.F. 1981. Potency testing of fish vaccines. In: Anderson, D.P. and Hennessen, H. (Eds).Fish Biologies: Serodiagnostics and Vaccines. Devel. Biol. Stand., 49: 447- 454

6. Andrews, S. R., N. P. Sahu, A. K. Pal, S.C. Mukherjee and S. Kumar. 2011. Yeast extract, brewer's yeast and spirulina in diets for *Labeo rohita* fingerlings affect haemato-immunological responses and survival following *Aeromonas hydrophila* challenge. Res. Vet. Sci. 91: 103-109

7. Brown, M. E. 1957. Experimental Studies on Growth in Fish Physiology, M. E. Brown (Ed). New York, Acad. Press, 1: 361-400.

8.Collins, M.D. and G.R Gibson. 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. J. American Clinic. Nutr. 69: 1052–1057.

9. Denev, S., Y. Staykov, R. Moutafchieva and G. Beev. 2009. Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture. Inter. Aqua. Res. 1:1-29.

10. Fadhal, A.A. and S.A. Mustafa. 2020. Influence of phytase enzyme on growth performance and survival rate challenge with *Saprolegnia spp.* in common carp. Iraqi J. Agric. Sci. 51 (5): 158-1465.

11. FAO. Food and Agriculture Organization of the United Nations. 1981. Report of the Symposium on New Development in the Utilization of Heated Effluent and of Recirculation System for Intensive

Aquaculture. Stavanger, 29-30 May 1980. Rome, EIFFAC/ T39

12. Firouzbakhsh, F., Z. Mehrabi, M. Heydari, M. K. Khalesi and M. A. Tajick. 2014. Protective effects of a synbiotic against experimental *Saprolegnia parasitica* infection in rainbow trout (*Oncorhynchus mykiss)*. Aqua. Res. 45(4): 609-618

13. Hoseinifar, S.H. E. Ringø, A.S. Masouleh and M.A. Esteban. 2014. Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: Reviews in Aquaculture, 6: 1- 14

14. Manning, T.S. and G.R. Gibson, 2004. Prebiotics. Best Practice and Research Clinical Gastroenterology, 18: 287-298

15. Maroni, K. 2000. Monitoring and regulation of marine aquaculture in Norway. Appl. Ichthyol. 16: 192–195.

16. Mehrabi, Z. F. Firouzbakhsh and A. Jafarpour. 2011. Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss)* fingerlings. J. Anim. Physiol. Anim. Nutr.24: 0396–1439

17. Merrifield, D.L. G. Bradley, R.T.M. Baker and S.J. Davies 2010. Probiotic applications for rainbow trout (*Oncorhyncus mykiss)* II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria post antibiotic treatment. Aqua. Nutr.1,16: 496–503

18. Mustafa, S. A. A. J. Al-Rudainy and J. K. Al-Faragi. 2019. Assessment of hydrogen peroxide on histopathology and survival rate in common carp, *Cyprinus carpio* L. Infected with Saprolegniasis. Iraqi J. Agric. Sci. 50 (2):697-704

19. Nayak, S.K. 2010. Probiotics and immunity: a fish perspective. Fish and Shellfish Immunol. 29: 2-14

20. Pandiyan, P. D. Balaraman, R. Thirunavukkarasu, E.G.J. George, K. Subaramaniyam and S. Manikkam. 2013. Probiotics in aquaculture. Drug Inven. Today, 5: 55-59

21. Roberfroid, M.B. 1998. Prebiotics and synbiotics: concepts and nutritional properties. British J. Nut. 80: 197–202

22. Rodriguez-Estrada, U. S. Satoh, Y. Haga, H. Fushimi, and J. Sweetman. 2009. Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout (*Oncorhynchus mykiss*). Aqua. Sci. 57: 609–617

23. Sahu, M.K. N.S. Swamakumar, K. Sivakumar, T. Thangaradjou, and L.Kannan. 2008. Probiotics in aquaculture: importance and future perspectives. Indian J. Microbiol. 48: 299–308

24. Schmalhusen, L.1926. Studien Uber Washstum and Diffrentzieung. III. Die Embryonalwachstum Skurvedes Hiichen. Wilhem Roux arch, entwic klungsmech. Org, (cited by It oar, W. S., J. Randall and J.R. Brett. Fish PhysiologyVIII), 322-387

25. Siwickiet, A.K. D.P. Anderson and G.L. Rumsey. 1994. Dietary intake of Immunostimulants by rainbow trout effects non-specific immunity and protection against furunculosis. Vet. Immunol. Immunopathol. 41: 125-139

26. Staykov, Y., P. Spring, S. Denev and J.Sweetman 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aqua. Int. 5(2):153–161

27. Sun, Y.Z. H.L. Yang, R.L. Ma, K. Song and J.S. Li. 2011. Effect of *Lactococcus lactis* and *Enterococcus faecium* on growth performance, digestive enzymes and immune responseof grouper *Epinephelus coioides*. Aquac. Nutr. 18: 281–289.

28. Talpur, A.D. and M. Ikhwanuddin 2013. Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunol. 34:254–264.

29. Talpur, A.D. M.A. Munir, A. Mary and R. Hashim. 2014. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. Fish Shellfish Immunol. 39: 34-41

30. Thrall, M. A., D. C. Bker, T. W. Campbell, D. Dinicola, M. J. Fettman, E. D. Lassen, A. Reber and G. Weiser. 2006. Veterinary hematology and clinical chemistary.

Lippincott Williams, Philadelphia PP. 214 - 219.

31. Uten, E. 1978. Standard Methods and Terminology in Fin Fish Nutrition and Fish Feed Technology. Hamburg. 20-23. June vol. 111. Berlin, 1979

32. Wang, Y.B. and Z. Xu. 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Tech. 127: 283–292

33. Wang, Y. B., Z. Tian, J. Yao and W. Li. 2008. Effect of probiotics, *Enterococcus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. Aquaculture, 277: 203–207

34. Welker, T.L. C. Lim, M. Yildirim-Aksoy , R. Shelby and P.H. Klesius. 2007. Immune response and resistance to stress and *Edwardsiella ictaluri*, fed diets containing commercial whole-cell yeast or yeast subcomponents. J. World Aqua. Soc. 38(1): 24–35

35. Ye, J.D. K.Wang, F.D. Li and Y.Z. Sun.2011. Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder Paralichthys olivaceus. Aquac. Nutr. 17: 902-911.

36. Zhang, C. N. X.F. Li, W. N. Xu, G. Z. Jiang, K.L. Lu, L.N. Wang and W.-B. Liu, 2013. Combined effects of dietary fructooligosaccharide supplementation and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*). Fish Shellfish Immunol. 35: 1380–1386.