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Research Article
Effect of Different Growth Promoters on Growth Performance, Feed Utilization and Body Composition of Common Carp (*Cyprinus carpio*)

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Abstract
Objective: This study was carried out to compare the effect of some commercial growth promoters (Probiotic, prebiotic and acidifier) on growth performance, feed utilization, body composition and blood pictures of juveniles common carp (*Cyprinus carpio*) reared in earthen ponds. **Methodology:** A total number of (3600) apparently healthy juveniles common carp (20 g) reared in 12 earthen ponds (100 m^3 each) to assign four treatments (Control, probiotic (Biogen®), prebiotic (Garlin Extra4®) and acidifier (Galliacid®)) in triplicate for each treatment. The fish fed 3% of their body weight twice a day for 183 days. **Results:** The results indicated that fish performance parameters were superior significantly (p>0.05) in probiotic treatment followed by prebiotic then acidifier finally control group. The same trend was observed in feed utilization parameters. In body composition analysis the best protein content was observed in probiotic treatment but the highest fat content was in acidifier and there were significant differences in ash content. There were significant differences in total white blood cells count as indicator for immune response. **Conclusion:** These results suggested that supplementing diets with commercial feed additives promotes growth performance, feed utilization and net financial return comparing with the control, but the comparison between them showed that probiotics was more superior followed by prebiotics then acidifier in juveniles common carp diets at practical applied field.

Key words: Probiotics, prebiotics, acidifier, common carp

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Carp has been found as the dominate fish species in the world aquaculture production. In 2008, carp fish recorded the highest outcome value as a major species group. The total production of the common carp in the world is around 4 million ton (3877118 kg in 2012)[1].

Because of the rapid industrialization of carp production, attention must be paid to develop diet formulation to support the economical and environmental problems facing the fish culture.

It is well established in the field of aquaculture that the use of antibiotic as feed additives could improve growth performance. However, in connection with the ban of antibiotic as growth promoters by the European Union in 2006, according to Ringo *et al.*[2], new strategies in feeding and health management in fish aquaculture practice have received much attention[3].

There has been heightened research study to develop new dietary supplementation strategies in which various health and growth promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated[4].

Probiotics in aquaculture have been reported to provide beneficial effects[3]. The positive effects of probiotic administration to fish growth and immune response are well-documented[5,6]. Probiotics may promote growth, non-specific immune response, disease resistance and the survival rate of aquatic animals[7-9].

Prebiotics is a non viable food component that confers a health benefit on the host associated with modulation of the microbiota[10]. They are important for improving growth performance, immunomodulation and resistance to diseases against well-known pathogens as well as the effect on gut microbiota of various fish species, shrimp and other aquatic organisms[11-14].

Acidifiers are a term, which describes the organic acids and salts[15]. A number of studies on different fish species indicated that a range of organic acids and their salts or mixtures can improve growth, feed utilization and disease resistance in fish. Dietary acidifiers have been reported as beneficial in aquaculture where they confer benefits such as improved feed utilization, growth and resistance to bacterial pathogens[16]. There has been an increasing interest on the use of acidifiers in aquaculture due to the removal of antibiotic growth promoters by the European Union in 2006. Research study regarding acidifires showed different results due to using different kinds of acidifires[15,17].

This study aimed to compare between the effect of some commercial growth promotors (Probiotic, prebiotic and acidifier) on growth performance, feed utilization, body composition and some blood parameters of juveniles common carp (*Cyprinus carpio*) reared in earthen pond.

MATERIALS AND METHODS

An over-winter 3600 juveniles common carp (20 g) were used in the present study, representing 4 treatments (Control, probiotic, prebiotic and acidifier) at three groups of fish for each were stoked in 12 earthen pond each (100 m^3) At a density of 3 fish m^{-3}, at EL-Zawya fish farm, Kafr EL-shaikh Governorate, belonging to the general Authority for fish Resources Development (GAFRD), Ministry of Agriculture and land Reclamation, Egypt. The feeding experiment lasted for 6 months (183 days). The fish were acclimated on the culture system for 2 weeks. A random sample of 20 fish at the beginning and at the end of the experiment from each pond was taken, weight collectively and stored at -20°C for initial and final body composition analysis.

Water quality parameters: Including water Temperature (T), Dissolved Oxygen (DO) and pH were monitored weekly. The average values of these parameters throughout the study were: $T = 26.5\pm1°C$, $DO = 7.3\pm1.2$ mg L^{-1} and $pH = 7.75\pm0.20$. They were appropriate for common carp cultivation during the whole experiment period.

Test diets and feeding regime: A commercial basal diet was used (control group) containing 25% crude protein, 5.79% ether extract, 6.70% crude fiber, 14.2% ash, 49.41% NFE and 3698 kcal kg^{-1} GE supplemented with the different commercial growth promoters probiotic (Biogen®) containing (Allicin, high-unit hydrolytic enzymes, *Bacillus subtilis*, amylase, protease, lipase and garlic powder) prebiotic (Garlen extra 4®) containing (Garlic extract and blend of volatile oils) and acidifier (Galliacid®) containing (Fumaric acid, calcium format, calcium propionate, potassium sorbate and hydrogenated vegetable oil) representing 4 treatments (control, probiotic, prebiotic and acidifier). They added in the diets at the recommend level of the producers (2, 0.2 and 0.6 kg t^{-1} diet for probiotic, prebiotic and acidifier, respectively). Feeding level was 3% of the total biomass until the end of the experiment. The test diets were fed to the fish twice a day (9 am and 2 pm). Daily rations were readjusted each 15 days interval according to the new average weights until the end of the feeding period.

Viability test: The viability test of the probiotic (Biogen) was carried out before using according to the method outline by Martin *et al.*[18]. The viable contents was determined by containing Colony Forming Unit (CFU), which is considered an indication for the viability of the microorganisms present viable in this commercial probiotic and so represents its growth promoting effect determination showed the presences of (6×10^6) CFU for the commercial probiotic (BIOGEN©).

Immune response of fish as affected by treatments: At the end of the experiment a number of fish were subjected to the differential count of white blood cells. Blood films was staining by Giemsa's stain method Sakai *et al.*[19]. Blood films were used to determine the number of leucocytes, being indicator for immune response for juveniles feeding on different types of natural growth promoters.

Chemical analysis of fish body: At the beginning and the end of the experiment, a sample of fish from each pond were performed on a pooled sample, which was weighed and frozen at -20°C for final body composition analysis. Moisture, protein, lipid and ash were performed according to the standard methods[20].

Calculations of fish performance: Growth performance and feed efficiency parameters were calculated as follows:

$$\text{Weight Gain (WG)} = \text{Wf-Wi}$$

$$\text{Specific Growth Rate (SGR)} = (\text{LnWf-LnWi})\times\frac{100}{t}$$

where, Wi and Wf are initial and final weights (g), t is duration of experimental (days) and ln is the natural logarithm.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Dry feed fed (g)}}{\text{Fish weight gain (g)}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}\times100$$

$$\text{Protein Productive Value (PPV\%)} = \frac{\text{Protein gain (g)}}{\text{Protein fed (g)}}\times100$$

$$\text{Energy Retention (ER)} = \frac{\text{Retained energy in carcass (kcal)}}{\text{Energy intake (kcal)}}\times100$$

Statistical analysis: Statistical analysis was carried out using one-way analysis of variance using SPSS (version 16) for Windows (SPSS, Chicago, IL, USA)[21]. Differences between means were determined using Duncan's multiple test ($p<0.05$)[22].

RESULTS AND DISSCUSION

Average values of initial body weight, final body weight, weight gain and specific growth rate of common carp fed different growth promoters diets are given in Table 1. The data in Table 1 showed that initial weight was nearly similar in all treatment groups with no significant differences ($p<0.05$). The data showed also that there were significant differences ($p>0.05$) among all treatments in final body weights (FW), Weight Gain (WG) and Specific Growth Rate (SGR). Probiotic treatment recorded the highest final body weight, weight gain and specific growth rate (919.899 and 3.73), respectively followed by prebiotic (746, 726 and 3.61) then acidifier (664, 644 and 3.55). All tested diets were superior as compared with the control diet with no supplementation (552, 532 and 3.45 g). The present results were in agreement with the results obtained by many researchers. Renuka *et al.*[23] suggested that the incorporation of probiotic in common carp diets stimulated fish growth and digestion as micro biota colonization enzymes that hydrolyze complex molecules, facilitate better digestion and absorption of macronucleus resulting in higher protein and energy deposition in the body tissues. In these aspects[24], pointed out to the improvement of digestion and metabolism in the fish body due to the presence of the bacillus in the probiotic Biogen, moreover the prevention of pathogenic

Table 1: Growth performance parameters of common carp fed the tested diets

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	Specific growth rate
Control	20.00	552 ± 0.06^d	532 ± 0.06^d	3.45 ± 0.00^d
Probiotics	20.00	919 ± 0.32^a	899 ± 0.32^a	3.73 ± 0.00^a
Prebiotics	20.02	746 ± 0.10^b	726 ± 0.10^b	3.61 ± 0.00^b
Acidifier	19.98	664 ± 0.30^c	644 ± 0.30^c	3.55 ± 0.00^c

Values in the same column with different superscripts are significantly different at $p<0.05$

bacteria colonies in fish gut. Faramarzi *et al.*[25] were in accordance with the results obtained in the present study, where they found that the addition of 0.1% probiotics (*Bacillus subtilis* c-3102 spores) in common carp fry diets improved fish growth and mitigated the effects of stress factors. In this particular, diets supplemented with *Bacillus subtilis* c-3102 spores resulted in improving growth performance of koi carp significantly than those fed the control basal diets.

The positive effect of probiotic was also observed in several kind of fish, in seabream (*Sparus aurata* L.)[26,27] and large croaker (*Larimichthys crocea*)[28]. The high viability *Bacillus subtilus* may be considered as another reason for the positive and better effect of Biogen. This fact was proved by the studies carried out by Mohapatra *et al.*[29], who found that incorporation of live probiotic microorganisms (*Lactobacilis lactis* and *Bacillus subtilus*) resulted in maximum growth performance in rohu (*Labeo rohita*) fingerlings in comparison with some combinations of inactivated probiotics. Other similar results were also observed for *Tilapia nilotica*[30], *L. rohita*[31] and *Cyprinus carpio*[32].

The present results indicated that prebiotics had the second superior effect in the diet of common carp in growth performance parameters. The differences in these parameters between either the control diet or the probiotic diets were significant. The fish fed diet supplemented with prebiotic showed improvement in fish growth as compared with the control diet, but this improvement was leaser than that in probiotics diets. It was suggested that the improvement in growth parameters occurred with the prebiotic diets may be due to the fermentation of prebiotics in colon, which promote the growth of the bacterial populations associated with the healthy well functioning colon. Beneficial types of colonic bacteria have the ability of oligosaccharides fermentation which are not used effectively by potentially pathogenic bacteria species[33]. As in the case of probiotics, the positive effect of prebiotics on growth was also found in different fish species such as *Atlantic cod*[34], *Turbot larvae*[35], *Rainbow trout*[36,37] *Atlantic salmon*[38] and *Hybrid tilapia*[39].

Concerning acidifier, the results showed its superiority in increasing growth over the control diet. These results were in accordance with the results obtained by Ng *et al.*[40] who found that the dietary organic acids can exert strong anti-microbial effects and have the potential to exert beneficial effects on growth, nutrient utilization and disease resistance in tilapia when tested in hyprid tilapia (*Oreochromis* sp.) which may be a reflection of the reduction of pH in the digesta in the stomach and gut. This was indicated by the significant reduction of total bacteria per gram in the feaces of fish fed diets supplemented with organic acids. Other investigators studied the effect of organic acid salts or salts blend (calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid) on growth of tilapia at different levels and found that these salts and blends may be especially useful during grow out period in tilapia culture. Baruah *et al.*[41] observed that citric acid of microbial phytase have a synergistic effect on the bioavailability which was more pronounced in low-protein diets. Some researchers proved the ability of potassium diformate in controlling *Vibrio anguillarum* in tropical tilapia culture[42]. In this respect, higher cumulative mortality of fish fed diets was obtained with no organic acids as composed with those received diets supplemented with organic acid after 16 days past challenge with *Streptococcus agalactiae*[40].

Efficiency of feed and protein utilization: Feed and protein utilization parameters expressed as FCR, PER, PPV and ER were illustrated in Table 2. Data obtained showed that there were significant differences (p>0.05) among the different treatments in FCR. The best value (2.28) was recorded in probiotic diets and there was no significant difference between fish received prebiotic and acidifier diets. The differences between probiotic treatment and both the control or prebiotic and acidifier were significant.

Concerning PER, the results indicated a significant difference (p>0.05) between the control (1.79) and the other tested treatments, where the tested supplemented diets with probiotic, prebiotic or acidifier were significantly better than the control. On the other hand there were no significant differences among the tested diets (1.83, 1.83 and 1.83).

Protein productive value (PPV%) showed significant differences between the control diets and all the tested diets probiotic, prebiotic, acidifier (19.18, 32.81, 27.58 and 22.79) respectively except acidifier diets. PPV% was superior in probiotic diets than the other treatments. At energy retention (ER) there were significant differences (p>0.05) between all

Table 2: Feed utilization parameters of common carp fed tested diets

Treatments	FCR	PER	PPV	ER
Control	2.34 ± 0.03^{a}	1.79 ± 0.03^{b}	19.18 ± 1.66^{c}	13.47 ± 1.12^{d}
Probiotics	2.28 ± 0.00^{c}	1.83 ± 0.00^{a}	32.81 ± 0.66^{a}	18.63 ± 0.80^{a}
Prebiotics	2.29 ± 0.00^{b}	1.83 ± 0.00^{a}	27.58 ± 1.85^{b}	16.57 ± 0.93^{b}
Acidifier	2.30 ± 0.00^{b}	1.83 ± 0.00^{a}	22.79 ± 1.17^{c}	14.96 ± 0.33^{c}

Values in the same column with different superscripts are significantly different at p<0.05, FCR: Feed conversion ratio, PER: Protein efficiency ratio, PPV: Protein production value, ER: Energy retention treatments and the control one. The highest value recorded for probiotic (18.63) then prebiotic (16.57) followed by acidifier (14.96), the lowest value recorded in control group (13.47).

The obtained data were in the same trend found in the previous studies about the efficiency of feed utilization, where several researchers recorded different degrees of improvement in feed and protein utilization parameters in diets supplemented with probiotic or growth promoters, which reflected the increasing growth rate[23,43]. Faramarzi *et al.*[25] found also improvement in the feed utilization in common carp fed diet supplemented with 0.1% probiotics (*Bacillus subtilis* c-3102 spores). Similar positive effects in feed utilization were recorded in many fish species as mentioned previously during the discussion of growth performance.

From the economical stand point of view, the results declared that all diets treated with feed additives, commercial probiotic, prebiotic and acidifier which little costly compared with control basal diet. But the return in high growth performance and feed utilization parameters showed that probiotic treatment achieved the highest net financial return, followed by prebiotic, acidifier and control.

Body composition: Body composition of common carp fed different growth promoters are presented in Table 3. Statistical analysis of these data showed no significant difference ($p<0.05$) among all treatments at the end of the experiment in DM. Meanwhile the other parameters (CP, EE and ash) declared that there were significant differences ($p>0.05$) between all treatments. The highest CP found at carcass for probiotics treatment (62.09) then prebiotics (57.65). However, acidifier more superior (50.50) than the control (41.80) but it is lowest than the values at the beginning.

According to EE the final carcass there were significant differences between treatments the highest one recorded in acidifier (23.53) then probiotics (20.17) followed by control and prebiotics (22.20 and 21.87), respectively with no significant differences. The ash content of fish carcass was higher in fish treated with prebiotics and acidifier with no significant differences (10.70 and 10.12) and the same observed in control and probiotics (6.86 and 5.85).

The improvement in body composition of carp fed probiotic is a significant evidence of the improvement in general health condition of the reared fish. These positive effect in body composition of common carp may be due to improving of growth performance, enhance the metabolism and energy of fish body cells and raise the efficiency of feeds[44]. The results of body composition in this study were in close agreement with Mohamed *et al.*[45] for tilapia. On the other hand Eid and Mohamed[46] found no statistical differences were observed in whole body moisture, crude protein, ether extract and ash for mono sex *O. niloticus* fingerlings fed diets containing different levels of commercial feed additives.

Immune response: Determining the immune response of experimental fish fed different diets containing natural growth promoters had been done by differential count of blood film. The objective of that is to assess the changes in white blood cells following spontaneous stress factors surrounding the common carp juveniles reared in earthen ponds in order to evaluate the serosity of the disease and other stress factors on the basis of blood alterations, which reflect the immune response are presented in Table 4.

The results found that there were significant differences in total count of white blood cells, the fish fed with control and acidifier group have highest total count (35.67 and 35.33) followed by prebiotic (33.33), the last one is probiotics (31.67).

By differentiation, no significant differences were observed in heterocytes, lymphocytes, monocytes, esenocytes and basophiles.

Table 3: Body composition on dry matter basis of common carp fed the tested diets

Treatments	DM	Crude protein	Ether extract	Ash
Initial	17.00	54.63	16.18	11.4
Control	25.41±1.85	41.80±0.90^{d}	22.50±1.40^{ab}	6.86±0.14^{b}
Probiotics	28.50±0.98	62.09±0.88^{a}	20.17±1.01^{b}	5.85±0.41^{b}
Prebiotics	25.83±1.36	57.65±1.12^{b}	21.87±0.69^{ab}	10.70±0.44^{a}
Acidifier	24.46±0.59	50.50±0.87^{c}	23.53±0.52^{a}	10.12±0.08^{a}

Values in the same column with different superscripts are significantly different at $p<0.05$

Table 4: Blood film analysis for common carp fed the tested diets

	Differential count of white blood cells (1×10^{3} cell μL^{-1})					
Treatments	Total	Heterocytes	Lymphcytes	Monocytes	Esenocytes	Basophiles
Control	35.67±0.88^{a}	10.00±0.58^{a}	82.67±0.66^{a}	5.67±0.88^{a}	1.00±0.58^{a}	0.67±0.33^{a}
Probiotics	31.67±1.20^{ab}	9.33±0.88^{a}	85.00±0.57^{a}	4.67±0.88^{a}	0.33±0.33^{a}	0.66±0.33^{a}
Prebiotics	33.33±1.20^{b}	11.00±3.06^{a}	82.00±2.65^{a}	5.33±0.88^{a}	1.00±0.58^{a}	0.66±0.33^{a}
Acidifier	35.33±1.20^{a}	8.00±1.15^{a}	83.66±1.45^{a}	6.00±0.58^{a}	0.67±0.33^{a}	1.66±0.33^{a}

Values in the same column with different superscripts are significantly different at $p<0.05$, the tested diets

Haematological parameters are considered as proper indices for tracking health status of fish and their response to environmental stresses[47]. The WBC (leucocytes) are one of the most important cells that can stimulate immune responses of fish and serves as one of the 1st line of body defence and their numbers increase sharply when infections arise[48]. Also, these cells produce antibody and can perform macrophagus activity[49]. The total WBC in this experiment indicated that all treatments were within the normal range which reflect the strength of immunity especially in the control and acidifier treatments.

CONCLUSION

This study indicates that common carp fed diets with different natural growth promoters may improve growth performance, feed utilization, body composition and net financial return in common carp juveniles diets than the control. But when comparing among the three sources the probiotic was the superior, followed by prebiotic then acidifier in practical filed.

REFERENCES

1. FAO., 2014. Global aquaculture production 1950-2012. Food and Agricultural Organization, Rome, Italy.
2. Ringo, E., Z. Zhou, J.L.G. Vecino, S. Wadsworth and J. Romero *et al*., 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?. Aquacult. Nutr., 22: 219-282.
3. Balcazar, J.L., I. de Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell and J.L. Muzquiz, 2006. The role of probiotics in aquaculture. Vet. Microbiol., 114: 173-186.
4. Denev, S.A., 2008. Ecological alternatives of antibiotic growth promoters in the animal husbandry and Aquaculture. DSC. Thesis, Department of Biochemistry Microbiology, Trakia University Stara Zagora Bulgaria.
5. Nikoskelainen, S., A.C. Ouwehand, G. Bylund, S. Salminen and E.M. Lilius, 2003. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish Shellfish Immunol., 15: 443-452.
6. Kumar, P., N.P. Sahu, N. Saharan, A.K. Reddy and S. Kumar, 2006. Effect of dietary source and level of chitin on growth and survival of post-larvae *Macrobrachium rosenbergii*. J. Applied Ichthyol., 22: 363-368.
7. Newaj-Fyzul, A., A.A. Adesiyun, A. Mutani, A. Ramsubhag, J. Brunt and B. Austin, 2007. *Bacillus subtilis* AB1 controls aeromonas infection in rainbow trout (*Oncorhynchus mykiss*, Walbaum). J. Applied Microbiol., 103: 1699-1706.
8. Ringo, E., A. Dimitroglou, S.H. Hoseinifar and S.J. Davies, 2014. Prebiotics in Fin Fish: An Update. In: Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics, Merrifield, D. and E. Ringo, (Eds.). Wiley-Blackwell Publishing, Oxford, UK., pp: 360-400.
9. Hauville, M.R., J.L. Zambonino-Infante, J.G. Bell, H. Migaud and K.L. Main, 2016. Effects of a mix of *Bacillus* sp. as a potential probiotic for Florida pompano, common snook and red drum larvae performances and digestive enzyme activities. Aquacult. Nutr., 22: 51-60.
10. FAO., 2007. The state of world fisheries and aquaculture 1998. Food and Agriculture Organization of the United Nations, Rome, Pages: 112.
11. Ganguly, S., K.C. Dora, S. Sarkar and S. Chowdhury, 2013. Supplementation of prebiotics in fish feed: A review. Rev. Fish Biol. Fish., 23: 195-199.
12. Torrecillas, S., D. Montero and M. Izquierdo, 2014. Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action. Fish Shellfish Immunol., 36: 525-544.
13. Song, S.K., B.R. Beck, D. Kim, J. Park, J. Kim, H.D. Kim and E. Ringo, 2014. Prebiotics as immunostimulants in aquaculture: A review. Fish Shellfish Immunol., 40: 40-48.
14. Eshaghzadeh, H., S.H. Hoseinifar, H. Vahabzadeh and E. Ringo, 2015. The effects of dietary inulin on growth performances, survival and digestive enzyme activities of common carp (*Cyprinus carpio*) fry. Aquacult. Nutr., 21: 242-247.
15. Luckstadt, C., 2008. The use of acidifiers in fish nutrition. CAB Rev.: Perspect. Agric. Vet. Sci. Nutr. Nat. Res., 3: 1-8.
16. Luckstadt, C., 2007. Effect of Organic Acid Containing Additives in Worldwide Aquaculture-Sustainable Production the Non-Antibiotic Way. In: Acidifiers in Animal Nutrition-A Guide for Feed Preservation and Acidification to Promote Animal Performance, Luckstadt, C. (Ed.). Nottingham University Press, Nottingham, pp: 71-77.
17. Luckstadt, C., 2006. Use of organic acids as feed additives-sustainable aquaculture production the non-antibiotic way. Int. Aquafeed, 9: 21-26.
18. Martin, P.A., J.R. Lohr and D.H. Dean, 1981. Transformation of *Bacillus thuringiensis* protoplasts by plasmid deoxyribonucleic acid. J. Bacteriol., 145: 980-983.
19. Sakai, M., T. Otubo, S. Atsuta and M. Kobayashi, 1995. Enhancement of resistance to vibriosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum), by oral administration of *Clostridium butyricum* bacterin. J. Fish Dis., 18: 187-190.
20. AOAC., 1995. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
21. SPSS., 2007. Statistical Package for Social Science Version 16.0 Window. SPSS Inc., Chicago, USA.
22. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.

23. Renuka, K.P., M. Venkateshwarlu, A.T.R. Naik and S.M.P. Kumara, 2013. Influence of probiotics on growth performance and digestive enzyme activity of common carp (*Cyprinus carpio*). Int. J. Curr. Res., 5: 1696-1700.
24. Agouz, H.M. and W. Anwer, 2011. Effect of Biogen® and Myco-Ad® on the growth performance of common carp (*Cyprinus carpio*) fed a mycotoxin contaminated aquafeed. J. Fish. Aquat. Sci., 6: 334-345.
25. Faramarzi, M., S. Kiaalvandi and F. Iranshahi, 2011. The effect of probiotics on growth performance and body composition of common carp (*Cyprinus carpio*). J. Anim. Vet. Adv., 10: 2408-2413.
26. Salinas, I., A. Cuesta, M.A. Esteban and J. Meseguer, 2005. Dietary administration of *Lactobacillus delbrueckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. Fish Shellfish Immunol., 19: 67-77.
27. Avella, M.A., G. Gioacchini, O. Decamp, P. Makridis, C. Bracciatelli and O. Carnevali, 2010. Application of multi-species of *Bacillus* in sea bream larviculture. Aquaculture, 305: 12-19.
28. 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, 317: 155-161.
29. Mohapatra, S., T. Chakraborty, A.K. Prusty, P. Das, K. Paniprasad and K.N. Mohanta, 2012. Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: Effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquacult. Nutr., 18: 1-11.
30. Lara-Flores, M., M.A. Olvera-Novoa, B.E. Guzman-Mendez and W. Lopez-Madrid, 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 216: 193-201.
31. Ghosh, K., S.K. Sen and A.K. Ray, 2003. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. Israeli J. Aquacult., 55: 13-21.
32. Ramakrishnan, C.M., M.A. Haniffa, M. Manohar, M. Dhanaraj, A.J. Arockiaraj, S. Seetharaman and S.V. Arunsingh, 2008. Effects of probiotics and spirulina on survival and growth of juvenile common carp (*Cyprinus carpio*). Israeli J. Aquacult., 60: 128-133.
33. Yousefian, M. and M.S. Amiri, 2009. A review of the use of prebiotic in aquaculture for fish and shrimp. Afr. J. Biotechnol., 8: 7313-7318.
34. Refstie, S., T. Landsverk, A.M. Bakke-McKellep, E. Ringo, A. Sundby, K.D. Shearer and A. Krogdahl, 2006. Digestive capacity, intestinal morphology and microflora of 1-year and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. Aquaculture, 261: 269-284.
35. Mahious, A.S., F.J. Gatesoupe, M. Hervi, R. Metailler and F. Ollevier, 2006. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquacult. Int., 14: 219-229.
36. 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*). Aquacult. Int., 15: 153-161.
37. Yilmaz, E., M. A. Genc and E. Genc, 2007. Effects of dietary mannan oligosaccharides on growth, body composition and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. Israeli J. Aquacult., 59: 182-188.
38. Grisdale-Helland, B., S.J. Helland and D.M. Gatlin III, 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture, 283: 163-167.
39. Genc, M.A., E. Yilmaz, E. Genc, E. Genc and M. Aktas, 2007. Effects of dietary Mannan Oligosaccharides (MOS) on growth, body composition and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Israeli J. Aquacult., 59: 10-16.
40. Ng, W.K., C.B. Koh, K. Sudesh and A. Siti-Zahrah, 2009. Effects of dietary organic acids on growth, nutrient digestibility and gut microflora of red hybrid tilapia, *Oreochromis* sp. and subsequent survival during a challenge test with *Streptococcus agalactiae*. Aquacult. Res., 40: 1490-1500.
41. Baruah, K., N.P. Sahu, A.K. Pal, K.K. Jain, D. Debnath and S.C. Mukherjee, 2007. Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein level. Aquacult. Res., 38: 109-120.
42. Ramli, N., U. Heindl and S. Sunanto, 2005. Effect of potassium-diformate on growth performance of tilapia challenged with *Vibrio anguillarum*. Proceedings of the Annual Meeting of the World Aquaculture Society, May 9-13, 2005, Bali, Indonesia..
43. Lemieux, H., P. Blier and J.D. Dutil, 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? Fish Physiol. Biochem., 20: 293-303.
44. Mehrim, A.I., 2009. Effect of dietary supplementation of Biogen® (Commercial probiotic) on mono-sex *Nile tilapia Oreochromis niloticus* under different stocking densities. J. Fish. Aquat. Sci., 4: 261-273.

45. Mohamed, K.A., B. Abdel Fattah and A.M.S. Eid, 2007. Evaluation of using some feed additives on growth performance and feed utilization of monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. Agric. Res. J. Suez Canal Univ., 7: 49-54.
46. Eid, A. and K.A. Mohamed, 2008. Effect of using probiotic as growth promoter in commercial diets for monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-14, 2008, Cairo, Egypt, pp: 241-253.
47. Firouzbakhsh, F., F. Noori, M.K. Khalesi and K. Jani-Khalili, 2011. Effects of a probiotic, protexin, on the growth performance and hematological parameters in the Oscar (*Astronotus ocellatus*) fingerlings. Fish Physiol. Biochem., 37: 833-842.
48. Akrami, R., M. Nasri-Tajan, A. Jahedi, M. Jahedi, M.R. Mansour and S.A. Jafarpour, 2015. Effects of dietary synbiotic on growth, survival, lactobacillus bacterial count, blood indices and immunity of beluga (*Huso huso* Linnaeus, 1754) juvenile. Aquacult. Nutr., 21: 952-959.
49. Ta'ati, R., M. Soltani, M. Bahmani and A.A. Zamini, 2011. Effects of the prebiotics immunoster and immunowall on growth performance of juvenile beluga (*Huso huso*). J. Applied Ichthyol., 27: 796-798.