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Effects of Probiotic Aqualase on Kutum Fries (*Rutilus frisii kutum*) Growth and Immunity Characteristics

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Abstract: Kutum (*Rutilus frisii kutum*) is one of the important fish in the Caspian Sea and the Iranian Fisheries Organization (Shilat) produces up to 200 million fry (1–2 g b.w.) to restock the Caspian Sea population annually. The objective of this study was to investigate the effects of probiotic Aqualase (1, 1.5 and 2 g/kg of feed) on growth factors and Immunity Characteristics compared to control group (fish fed an unsupplemented probiotic in diet) in Kutum. After 8 weeks of feeding with the experimental diets, results showed an increase in weight, body length, survival rate, condition factor (CF) and Amylase, however the differences were not significantly with the control group ($P>0.05$). Others factor such as ALP, SGR, GR, as well as Immunity Characteristics C_3, C_4, IgM showed significant differences ($P<0.05$). These results indicate that Aqualase positively enhanced growth performance and immune response in Kutum, which means Aqualase increase potential of resistance of Kutum especially on handling during enrichment process. Also optimum level of dietary Aqualase is suggested to be 2 g/kg diet.

Key words: Probiotic Aqualase % Immunity Characteristics % *Rutilus Frisii Kutum* % SGR

INTRODUCTION

Fish is a principal source of animal protein for over half of the global population. Kutum, (*Rutilus frisii kutum* Kamenskii, 1901), Cyprinidae is an endemic fish of the Caspian Sea that live in the Caspian Sea near the coast, from the Terek River in the north to the southern part of the Caspian Sea. This is a very valuable commercial fish in the southern part of the Caspian Sea and has a great demand, due to its good taste and culinary customs of the local people. The average annual catch of Kutum in Iran was about 9600 tons in 1991-2001 [1] and more than 70% of caught fish by fisherman in Iran costal of Caspian Sea consist of this fish [2].

This species is a migratory anadromous fish spawning in rivers in March-April. It has a group synchronous, single spawning behavior, spawning on aquatic graveled, sandy substrates and weeds in rivers and lagoons. They migrate to the South river of Caspian Sea at spawning season [3]. During the last decade, water pollution, over fishing, reduction of river current in use of agriculture purpose, destruction of natural spawning beds has caused a negative impact and threatening on its natural population. To restock this valuable species in the Caspian Sea, the Iranian Fisheries Organization (Shilat) produces and releases up to 200 million fry (average weight 1 g) in to the Caspian Sea annually.

In the last decade, the scientific community carefully examined roles and effects of probiotics in aquaculture as an alternative to antimicrobial drugs, demonstrating positive effects on fish survival and growth [4], stress resistance [5], immunosystem enhancement [6] and finally general welfare [7]. Probiotics have a direct growth promoting effect on fish either by a direct involvement in nutrient uptake, or by providing nutrients or vitamins [8].

In restocking the Kutum to the Caspian Sea, fish at 1-3 gram weight are released to the sea. To increase the efficiency of releasing it is important to use

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supplementary material such as pro and prebiotics. In recent years there has been heightened research in developing dietary supplementation strategies in which various health promoting compounds have been evaluated in fish and other farmed animal [9]. Also several treatments are suggested to prevent losses of growth of fish [10]. The use of probiotics for disease prevention and improved nutrition in aquaculture is becoming increasingly popular due to an increasing demand for environment-friendly aquaculture. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods [11]. By using any additional or complement material, understand the effect of treatment is very important. So a great attention has been recently paid to biochemical characteristics of fish blood as an index of the state of internal milieu [12, 13]. The analysis of blood can reveal the internal biochemical changes, physiological condition and living habitat situation of fish. Also it can point up for us the pollutants, nutrition, stress and ecological conditions and in general the status of fish.

Studies on Kutum culture assist the aquaculture industry in meeting the ever increasing demand for Kutum, by improving production and enhanced survival of progeny. So, the present study was designed to evaluate the effect of probiotic Aqualase on some immune parameters and growth factors of Kutum fries.

MATERIALS AND METHODS

The experiments were performed at the Restocking Center of Shahid Rajaii Sturgeon Fish Farm, Sari, Iran. 2400 Kutum fingerlings used in this study are obtained from this farm, with average weight of 0.59±0.01 g were randomly distributed in 500 L fiberglass tanks and acclimatized to the experimental condition for 2 weeks feeding on a basal diet for adaptation. During the study the fish were fed with experimental diet three times for 8 weeks. Water temperature, Oxygen and pH ranged from 22.5-24.4°C, 8.1-8.7 mg/l and 7.9-8.3 respectively.

Diet preparation: Basal practical diet was formulated with estimated protein levels of 380g kg^{-1}. Twelve tanks with three replicates for each treatment group and control group (without any probiotic) were used with three levels (0.1%, 0.15% and 0.2%) of commercial probiotic Aqualase were added to the basal diet.

Growth and Feed Efficiency Parameters: The growth performance of fries such as, body weight increase (BWI), growth rate (GR), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF) and survival rate were calculated based on the standard formulae: BWI= (final body weight - initial body weight)×100/ initial body weight, GR= W2-W1/ T2- T1, SGR=(ln final weight-ln initial weight)×100/days, FCR = feed consumption / body weight gain, CF=(body weight/body length^3)×100 and survival rate=(final number of fish/initial number of fish)×100 [14-16].

The frozen samples were left to stand at room temperature to thaw and then inverted several times to mix. The serum samples for each specimen were analyzed together in one batch, to avoid run-to-run variability, for the following analyses: Complement C3, Complement C4, Immunoglobulin (IgM), Amylase, alkaline phosphatase. All analyses were performed using a blood chemistry Auto analyzer (Model Eurolyser).

To avoid dietary effect on metabolic status, fish were not fed 12 h before sampling. It was important in this study to gain reliable health status on the fish so as to avoid any distortion of the results by the diseased fish in the database. Accordingly, the following conditions had to be met to ensure this: absence of clinical signs of disease and of pathological and anatomical changes in the post-mortem examination [17].

Individual Kutum was rapidly netted and carefully placed in a circular, tank and were anesthetized with clove oil (0.5 ml/l). Fish were bleed cutting the tail, collecting the blood from the caudal vein. Serum was separated by centrifugation at 3000 rpm for 15 min at 4°C. After separation all sera maintained at -20°C on shore until processed in the laboratory.

Statistical Analysis: Growth factors and immunity characteristics of the Kutum were statistically evaluated using an analysis of variance procedure using one-way ANOVA. Differences in $P<0.05$ were considered to be significant and all results in the text were stated as mean ± standard division (SD).

RESULTS

The obtained results showed that treatment with Aqualase indicate high value than control for factors gain weight and length but this effect weren't significant ($p>0.05$). Also treatments with Aqualase could not influence significantly on CF, SR and FCR of Kutum ($p>0.05$), but it had influence on GR and SGR that was significant ($p<0.05$). The results of fish growth parameters are shown in Table1.

Table 1: Growth performance of Kutum fed by diets containing 3 levels of Aqualase for 8 weeks

Treatments	length	weight	CF	GR	SGR	SR	FCR
SFK+0.1% Aqualase	53.6 ±1.3 ^{a}	1.0±0.3^{a}	0.9±0.3^{a}	0.12±0.01^{ab}	0.62±0.4^{b}	86.2±12.3^{a}	1.8±0.4^{a}
SFK+0.15%Aqualase	52.7±7.5^{a}	0.8±0.1^{a}	0.9±0.2^{a}	0.23±0.04^{a}	0.94±0.3^{ab}	85.2±21.15^{a}	1.7±0.4^{a}
SFK+0.2% Aqualase	54.4±6.2^{a}	1.2±0.3^{a}	1.0±0.5^{a}	0.20±0.01^{b}	1.06±0.4^{a}	96.5±10.8^{a}	1.7±0.3^{a}
control	50.6±5.6 ^{a}	1.0±0.2^{a}	0.8±0.1^{a}	0.13±0.02^{ab}	0.69±0.4^{ab}	85.9±27.3^{a}	1.9±0.3^{a}

-Data are represented as Mean ± S.D. of three replicates.

-Numbers within the same row with different superscripts letters are significantly different (p<0.05).

Table 2: Immunity factors and enzyme activity performance of Kutum fed diets containing 3 levels of Aqualase for 8 weeks

Treatments	IgM	C_3	C_4	Amylase	ALP
SFK+0.1% aqualase	124.6±15.5^{ab}	55.8±7.3^{bc}	51.5±18.6^{ab}	131.5±17.9^{a}	51.4±10.4^{ab}
SFK+0.15%aqualase	125.2±16.9^{ab}	70.3±12.3^{a}	68.2±7.1^{a}	144.7±20.9^{a}	66.1±24.1^{a}
SFK+0.2% qualase	143.6±28.2^{a}	68.3±1.3^{ab}	75.4±14.6^{a}	140.8±22.7^{a}	73.4±15.8^{a}
control	106.7±25.1^{b}	51.1±5.2^{c}	39. 7±6.9^{b}	128.5±17.8^{a}	39.9±13.4^{b}

-Data are represented as Mean ± S.D. of three replicates.

-Numbers within the same row with different superscripts letters are significantly different (p<0.05).

In the present work, the rate of Igm, C3, C4 and ALP comparing to control and in most cases were significantly different (P<0.05), but not significant activity of amylase was observed in treatments with control group (p>0.05). The results of fish immunity parameters are shown in Table 2.

DISCUSSION

The larval period of Caspian kutum in artificial propagation is lasting two months in earthen pond feeding with artificial feed. In many fish species, the larval period is considered critical in the life history. Success of larval rearing depends mainly on the availability of suitable feed that is readily consumed and efficiently digested and that provides the required nutrients to support good growth and health [18]. In the present study the use of probiotic Aqualase has been introduced and the results revealed the fish in all treated groups received probiotic supplemented diets showed higher growth rate and resistance than control group.

The statistical analysis of growth performance of Kutum in the end of experimental period (Table 1) indicated an increase in growth rate (GR) and specific growth rate (SGR) in all groups that received probiotic. Pooramini *et al*. [19] reported that the use of Aqualase in rainbow trout (*Oncorhynchus mykiss*) diet is increased SGR in treated groups. Also Lim *et al*. [20] showed that naplius enriched with *Saccharomyces cerevisiae* in tilapia larvae diets is improved growth and length. Similar results have been reported for used of *S. cerevisea* in diets for carp [21] and Nile tilapia [22].

According to table 1, there weren't significant differences in weight gain and total length among treated groups and control (P>0.05) but group which received 0.20% of probiotic Aqualase was the higher compared with other groups. Condition factor (CF) didn't show significant differences between treatments receiving the diets supplemented with probiotic and control group as well. Similar study was done by Taghavi, [23] and Yousefian *et al.* [24] in using Aqualase on rainbow trout (*Oncorhynchus mykiss*) and Wild Carp (*Cyprinius Carpio*) respectively.

Survival rate of Kutum wasn't significantly higher in the all groups fed the Aqualase compared to the control group (P>0.05). The FCR values were decreased when compared to control fish but it was not significant (p>0.05). The best FCR was recorded in probiotic Aqualase 0.20% (1.67±0.28), suggesting that this yeast improved the utilization of feed. Similar results have been reported for *Saccharomyces cerevisiae* used in diets for carp Noh *et al.* [21] and Nile tilapia [22]. Probiotic treatment may be particularly useful to secure the settlement of fish intestinal microbiota and it may improve fish health [25]. According to Moriarty [26] beneficial bacterial cultures, added to water or fish feed, which can subsequently improve the health of the host can be defined as probiotic. The obtained results illustrate that using supplemented diets suggests that the addition of probiotic Aqualase improve the immunity of Kutum. The complement system is composed of more than 35 soluble plasma proteins that play key roles in innate and adaptive immunity [27]. The complement proteins have multifunctional roles in the defense against

microorganisms ranging from the opsonisation, lysis and killing of bacteria, to chemotaxis and anaphylaxis [28]. In the present study the value complement C_3 in the serum was increased in all treatments than control especially in group of more than 0.15% treatment. The most increase of complement C_4 was in 0.15% and 0.20% treatments. This result is similar to observations by Panigrahi *et al.* [29] that elevated levels of complement activity resulted from feeding *Lactobacillus rhamnosus* to rainbow trout (*Oncorhynchus mykiss*). Also Yousefian *et al*. [24] have shown that Aqualase increased the level of C_3 and C_4 complements in wild carp (*Cyprinius Carpio*).

Serum immunoglobulins are major component of the hemoral immune system and IgM is the main immunoglobulin present in fish Amount of IgM in the serum was increased in all treatments than to control especially in 0.20% treatment. Reyes-Beceril *et al*. [30] found that the addition of yeast in diet improved IgM levels of *Mycteroperca rosacea* exposed to stress. So it's possible to use Aqualase to increase resistance of Kutum against salt stress and during handling for enrichment. Also, Nayak *et al*. [31] reported that glucan of yeast improved production antibody of carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*.

As pointed by several authors the digestive enzyme like amylase could be improved by administration of probiotics to the diet [32, 33]. Essa *et al*. [34] observed *Saccharomyces cerevisiae* improved Nile Tilapia *Oreochromis niloticus* enzyme activities of amylase in the gastrointestinal tract and this probiotic showed a significant increase in amylase activity than the fish fed control diet. The present study showed that there isn't significant differences of amylase among treatments ($p>0.05$) but the lowest level of amylase was recorded in control group.

In the present study the alkaline phosphatase (ALP) in serum was increased in all treatments than to control especially in 0.20% treatment ($p<0.05$). Alkaline phosphatase exists to three forms (intestinal, bone and liver). Change of the level of this enzyme is depend on water chemical, food absorb, temperature, age [35] and compound phosphorous [36]. Result of Wilson *et al*. [37] on effect of *S. cerevisiae* as a feed additive on increase AP levels of rainbow trout (*Oncorhynchus mykiss*) and reports of Tovar-Ramirez *et al*. [38] on effect of live yeast in diet on improve digestive enzyme levels of Sea bass (*Dicentrachus labrax*) and result of Yousefian *et al*. [24] is in accordance with the result of the present study. To complete the effect of live organism in immune system of this fish using live feed of artemia and natural live feed of pond that showed (39 &40), its efficiency in rearing farmed fish is recommended.

CONCLUSION

Aqualase has potential of increase resistance and immunity factors of Kutum against pathogen bacteria and especially handling during releasing process to the Caspian Sea. As conclusion, the present study indicated that Aqualase positively enhanced growth performance, immune response and resistance in Kutum and optimum level of dietary Aqualase is 2 g/kg diet (0.20%).

REFERENCES

1. FAO, 2003. Fishstat Plus (v. 2.30) Fishery Statistics. FAO Fisheries Department, Rome, Italy.
2. Yousefian, M. and H. Mosavi, 2008. Spawning of south Caspian kutum (*Rutilus frisii kutum*) in most migratory river of south Caspian Sea. Asian Journal of Animal and Veterinary Advances, 3(6): 437-442.
3. Yousefian, M., 2011. Environmental factors influencing on migratory behavior of *Rutilus frisii kutum* in Shiroud River. World applied sciences Journal, 13(7): 1572-1579.
4. Burr, G., D. Gatlin and S. Ricke, 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. J. World Aquaculture Society, 36: 425-436.
5. Rollo, A., R. Sulpizio, M. Nardi, S. Silvi, C. Orpianesi, M. Caggiano, A. Cresci and O. Carnevali, 2006. Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiology and Biochemistry, 32: 167-177.
6. Picchietti, S., M. Mazzini, A.R. Taddei, R. Renna, A.M. Fausto, V. Mulero, O. Carnevali, A. Cresci and L. Abelli, 2007. Effects of administration of probiotic strains on GALT of larval gilthead seabream: immunohistochemical and ultrastructural studies. Fish and Shellfish Immunol., 22: 57-67.
7. Silvi, S., M. Nardi, R. Sulpizio, C. Orpianesi, M. Caggiano, O. Carnevali and A. Cresci, 2008. Effects of addition of *Lactobacillus delbrueckii* subsp delbrueckii on gut microbiota composition and contribution to the well-being of the European sea bass (*Dicentrarchus labrax* L.). Microbial Ecology in Health and Disease., 20: 53-59.

8. Ringo, E. and F.J. Gatesoupe, 1998. Lactic acid bacteria in fish: A review. Aquaculture, 160: 177-03.
9. Radfar, M. and P. Farhoomand, 2008. The role of probiotic and source of lactose as feed additives on performance and gut improvement in broilers. Asian Journal of Animal and Veterinary Advances, 3(3): 179-182.
10. Srinivasan, P., P. Ramasamy, G. P. Brennan and R.E.B. Hanna, 2007. Inhibitory effects of bacteriophages on the growth of *Vibrio* sp., pathogens of shrimp in the Indian aquaculture Environment. Asian Journal of Animal and Veterinary Advances, 2(4): 166-183.
11. Hrubec, T.C. and S.A. Smith, 2000. Hematology of fish. In: Feldman, B.F. Zinkl, J.G. Jain, N.C. (eds). Schalm's Veterinary Hematology. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins, pp: 1120-1125.
12. Yousefian, M., M. Sheikholeslami Amiri, M. Hedayatifard, A.A. Dehpour, H. Fazli, M. Ghiaci, S.V. Farabi and S.h. Najafpour, 2010a. Serum Biochemical Parameter of Male and Female Rainbow Trout (*Onchorhynchus mykiss*) Cultured in Haraz River, Iran. World Journal of Fish and Marine Sciences 2 (6): 513-518, ISSN 2078-4589 © IDOSI Publications, 2010.
13. Yousefian, M., M. Sheikholeslami Amiri, M. Hedayatifard, A.A. Dehpour, H. Fazli, M. Ghiaci, S.V. Farabi and S.H. Najafpour, 2010b. Serum biochemical parameter of male and female rainbow trout (*Onchorhynchus mykiss*) Cultured in Haraz River, Iran. World Journal of Fish and Marine Sciences 2 (6): 513-518, ISSN 2078-4589 © IDOSI Publications, 2010.
14. Hung, S.S.O., P.B. Lutes, A.A. Shqueir and F.S. Conte, 1993. Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*). Aquaculture, 115: 297-303.
15. Haghighi, D., T. Saad, C.R. Hosainzadeh, H. Sahafi and D. Mansouri, 2009. The effect of dietary lipid level on the growth of Kutum fry (*Rutilus frisii kutum*). Iranian Journal of Fisheries Sciences, 8(1): 13-24.
16. Luo, G., J. Xu, Y. Teng, C. Ding and B. Yan, 2010. Effects of dietary lipid levels on the growth, digestive enzyme, feed utilization and fatty acid composition of Japanese sea bass (*Lateolabrax japonicus*) reared in freshwater. Aquaculture Research, 41: 210-219.
17. Rehulka, J., B. Minarik and E. Rehulkova, 2004. Red blood cell indices of rainbow trout *Oncorhynchus mykiss* (Walbaum) in aquaculture. Aquaculture Research, 35: 529-546.
18. Waché Y., F. Auffray, F.J. Gatesoupe, J. Zambonino, V. Gayet, L. Labbé and C. Quentel, 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss* fry. Aquaculture, 258: 470-478.
19. Pooramini, M., A. Kamali, A. Hajomoradloo and M. Alizadeh, 2007. The effect of using yeast (*Saccharomyces cerevisiae* var. elipsoeidas) probiotic on growth parameters in rainbow trout (*Onchorhynchus mykiss*) larvae. International Training Course & Workshop Fish Nutrition & Diseases, pp: 48.
20. Lim, E.H., T.J. Lam and J.L. Ding, 2005. Single-Cell protein diet of novel recombinant vitellogenin yeast enhances growth and survival of first feeding tilapia (*Oreochromis mossambicus*) larvae. J. Nutr., 135: 513-518.
21. Noh, S.H., K Han, T.H. Won and Y.J. Choi, 1994. Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. Korean J. Anim. Sci., 36: 480-486.
22. Lara-Flores, M., M.A. Olvera-Novoa, B.E. Guzman-Méndez 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.
23. Taghavi, S., 2005. The effect addition probiotic of Thepax on factors of growth and survival on *Oncorhynchus mykiss* in growth stage. M.Sc Thesis in Fisheries, pp: 4-16.
24. Yousefian, M., M. Nikkhoo, R.Safari and M. Nikkhoo, 2010c. Evaluation of Aqualase as Probiotic on Wild Carp (*Cyprinius Carpio*) Growth, Immunity Characteristics and Resistance to Streptococcosis. Journal of Fisheries and Hydrobiology, 5(2): 173-178.
25. Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture, 180: 147-165.
26. Moriarty, D.J.W., 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture 164: 351-358.
27. Boshra, H., J. Li and J.O. Sunyer, 2006. Recent advances on the complement system in telost fish. Fish Shellfish Immunal., 20: 239-262.

28. Goldstein, I.M., 1988. Complement: Biologically active products. In: J.I. Gallin, I.M. Goldstein, Snyderman, R. (Eds.), Inflammation: Basic Principles and Clinical Correlates. Raven press, New York, pp: 55-74.
29. Panigrahi, A., V. Kiron, J. Puahqkaew, T. Kabayashi, S. Satoh and H. Sugita, 2005. The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 243: 241-254.
30. Reyes–Becerril, M., D. Tovar-Ramirez, F. Azcencio–valle, R. Civera-Cerecedo, V. Gracia-Lopez and V. Barbosa-Solomieu, 2008. Effect of dietary live yeast *Debaryomyces hansenii* on the immune and antioxiadant system in juvenile Leopard grouper (*Mycteroperca rosacea*) exposed to stress. Aquaculture, 280: 39-44.
31. Nayak, S.K., P. Swain and S.C. Mukherjee, 2007. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp (*Labeo rohita*). Fish Shellfish Immunol., 23: 892-896.
32. Yousefian, M. and A.M. Sheikholeslami, 2009. A review of the use of prebiotic in aquaculture for fish and shrimp. African Journal of Biotechnology, 8(25): 7313-7318.
33. Gomez, R., D. Geovanny and M.A. Shen, 2008. Influence of probiotics on the growth and digestive enzyme activity of White Pacific shrimp, *Litopenaeus vannamei*. Journal of Ocean University of China, 7: 215- 218.
34. Essa, M.A., S.S. EL-Serafy, M.M. El-Ezabi, S.M. Daboor, N.A. Esmael and S.P. Lall, 2010. Effect of different dietary probiotics on growth, feed utilization and digestive enzymes activities of Nile tilapia, *Oreochromis niloticus*. J Arabian aquaculture Society, 5(2): 143- 162.
35. Lemieux, H., P.U. Blier and J.D. Dutil, 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in Atlantic cod (*Gadus morhua*)? Fish physiol. Biochem., 20: 293-303.
36. Sknoberg, D.I., L. Yogev, R.W. Hardy and F.M. Dong, 1997. Metabolic response to dietary phosphorus intake in rainbow Trout (*Oncorhynchus mykiss*). Aquaculture, 157: 11-24.
37. Wilson, MR., E. van Ravenstein, N.W. Miller, L.W. Clem, D.L. Middleton and G.W. Warr, 1995. cDNA sequences and organization of IgM heavy genes in two holostean fish. Dev. Comp. Immunol., 19(2): 153-164.
38. Tovar-Ramireza, D., J. Zambiano C. Infante, Cahub, F.J. Gatesoupe and R. Vazquez-Jarez, 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus Labrax*) larval development. Aquaculture, 234: 415-427.
39. Yousefian, M. and S.H. Najafpour, 2011 Enrichment of Artemia Using Highly Unsaturated FattyAcid and Vitamin C in Larval Culture of *Acipenser persicus* World Applied Sciences Journal, 12(8): 1266-1268.
40. Yousefian, M., S.H. Najafpour, C. Makhtomi and G.D. Najafpour, 2010d. Live feed effects on growth rate of *Acipenser persicus* Brodin (1897) fingerling. World Applied Sciences Journal, 10(1): 29-32.