

EFFECTS OF MANNAN-OLIGOSACCHARIDE ON GROWTH, SURVIVAL AND DISEASE RESISTANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* LINNAEUS) FRY

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Abstract

Effects of mannan-oligosaccharide (MOS) on growth, survival and disease resistance of Nile tilapia (*Oreochromis niloticus* Linnaeus) fry were studied. Four concentrations of MOS at 0, 2, 4 and 6 g/kg feed were given to tilapia fry for 21 days. There were significant differences amongst average weight, length and average daily growth (ADG) of fry ($P < 0.05$) with the values of 0.78 ± 0.16 , 0.79 ± 0.19 , 0.85 ± 0.20 and 0.90 ± 0.19 g and 2.86 ± 0.24 , 2.88 ± 0.20 , 2.96 ± 0.25 and 3.03 ± 0.22 cm and 0.037 ± 0.007 , 0.037 ± 0.009 , 0.040 ± 0.010 and 0.042 ± 0.009 g/day respectively. Weight, length and ADG of tilapia fed with 4 and 6 g/kg MOS were significantly higher than the control ($P < 0.05$). There were no significant differences amongst feed conversion ratio (FCR) and survival rate of all experimental and control group ($P > 0.05$). Tilapia that were raised with MOS at all concentrations did show significantly higher resistance against pathogenic bacteria, *Streptococcus agalactiae* ($P < 0.05$) with mortality of 43.33 ± 20.82 , 3.33 ± 5.77 , 0.00 ± 0.00 and 0.00 ± 0.00 % for control, 2, 4 and 6 g/kg MOS respectively. These results indicated the benefit of MOS as feed supplement for tilapia fry in term of growth and disease resistance. Further study on the effects of MOS and its application in large tilapia will be conducted.

Keywords: Nile Tilapia, mannan-oligosaccharide, growth, survival, disease resistance

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INTRODUCTION

Nile tilapia (*Oreochromis niloticus* Linnaeus) has been the most common freshwater fish being cultured all around the world. In Thailand, this species count for more than 35% of the whole production from freshwater aquaculture. There are two main systems of tilapia culture in this country including pond and cage culture which require millions of fry each year. Cage culture can supply the better quality of the harvest especially for export. Fry production is the vital factor for tilapia culture. Many farmers prefer to use all-male tilapia fry because they can grow faster and eliminate the over-population problem. Farmers also can predict the production and harvesting

period. Besides the price issue, tilapia farmers also encounter with disease and growth problem. The most common disease of tilapia is caused by Gram positive bacteria namely, *Streptococcus agalactiae* (Darinya *et al.*, 2007). Streptococcosis is very common during summer season in conjunction with limited and poor quality water supply. Infected fish showed sluggish behavior, spiral swimming and protruded eyes (exophthalmia). These round-shaped gram positive bacteria can be isolated from internal organs and from the brain. Farmers are using licensed antibiotics to cope with the outbreaks but the results are still not satisfactory. Growth is another issue of interested for tilapia intensive cultures both in hatchery and grow-out stage. In hatchery, during sex-reversal period of 21 days, growth, size distribution and health appears to be the targets of research. In this report, mannan-oligosaccharide (MOS) was applied during the hatchery stage to determine the following parameters:

- growth and survival rate
- disease resistance against *S. agalactiae*

MATERIALS AND METHODS

Experimental Designs

Tilapia larva with just-disappeared yolk sac with average weight of 0.013 g were used for this experiment. The experiment was conducted in 100-L glass aquaria holding 80 liters of dechlorinated tap water. Each aquarium was stocked with 100 tilapia larva. Continuous aeration was applied throughout the trial. Powder feed (40% protein) was used as a basal diet.

This experiment was conducted by Completely Randomized Design with 4 experimental groups with three replicates as follows:

<u>Control</u>	Basal diet + 60 ppm 17 α -methyl testosterone (17 α -MT)
<u>Exp # 1</u>	Basal diet + 60 ppm 17 α -MT + 2 g/kg MOS
<u>Exp # 2</u>	Basal diet + 60 ppm 17 α -MT + 4 g/kg MOS
<u>Exp # 3</u>	Basal diet + 60 ppm 17 α -MT + 6 g/kg MOS

This experiment was designed according to the procedure of sex-reversal hatchery stage of Nile Tilapia in which 60 ppm of 17 α -MT will be applied for 21 days. Fish were fed 5 times a day between 8.00 am and 5.00 pm. Water were changed at 70% rate at every two days.

Determined Parameters

After 21 days of feeding trial, the following parameters were determined and statistically compared by Analysis of Variance and Duncan's New Multiple Range Test at $p = 0.05$ (Boonaom, 2007).

-Growth. Thirty percents of each replicate were measured for weight and length. Average daily growth (ADG) and feed conversion ratio (FCR) were also compared.

-Survival rate

-Disease resistance against pathogenic *Streptococcus agalactiae*. Ten fish from each replicate were challenged by immersion in 10^8 cfu/ml bacterial suspension for 6 hrs and transferred to normal water. Rate of mortality were observed and compared for 7 days. Bacterial isolation was performed from dead or moribund fish to confirm the cause of infection.

RESULTS

Growth and Survival

After 21 days of feeding trial, tilapia fry fed with 4 and 6 g/kg MOS showed significantly better weight, length and ADG than control and 2 g/kg ($P < 0.05$) (Table 1; Fig 1, 2 and 3). However, FCR were not significantly different ($P > 0.05$) (Table 1; Fig 4)

Table 1. Effect of different concentrations of mannan-oligosaccharide on weight, standard length, average daily growth and feed conversion ratio of Nile tilapia fry after an experimental period of 21 days (Mean \pm S.D.)

Treatment	Weight (g)	Standard Length (cm)	Average daily growth (g/day)	Feed conversion ratio
Control	0.78 \pm 0.16 ^a	2.86 \pm 0.24 ^a	0.037 \pm 0.007 ^a	1.42 \pm 0.10
2 g/kg	0.79 \pm 0.19 ^a	2.88 \pm 0.20 ^a	0.037 \pm 0.009 ^a	1.39 \pm 0.25
4 g/kg	0.85 \pm 0.20 ^b	2.96 \pm 0.25 ^b	0.040 \pm 0.010 ^b	1.28 \pm 0.19
6 g/kg	0.90 \pm 0.19 ^b	3.03 \pm 0.22 ^b	0.042 \pm 0.009 ^b	1.12 \pm 0.09

Note: Significant difference was indicated by different superscript letter within the same column at $P=0.05$

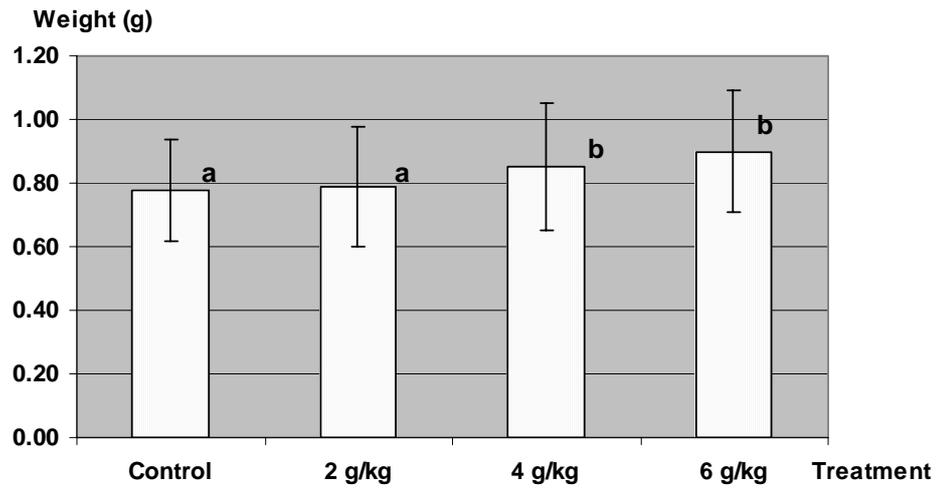


Figure 1. Effect of different concentrations of mannan-oligosaccharide on weight (Mean \pm S.D.) of Nile tilapia fry

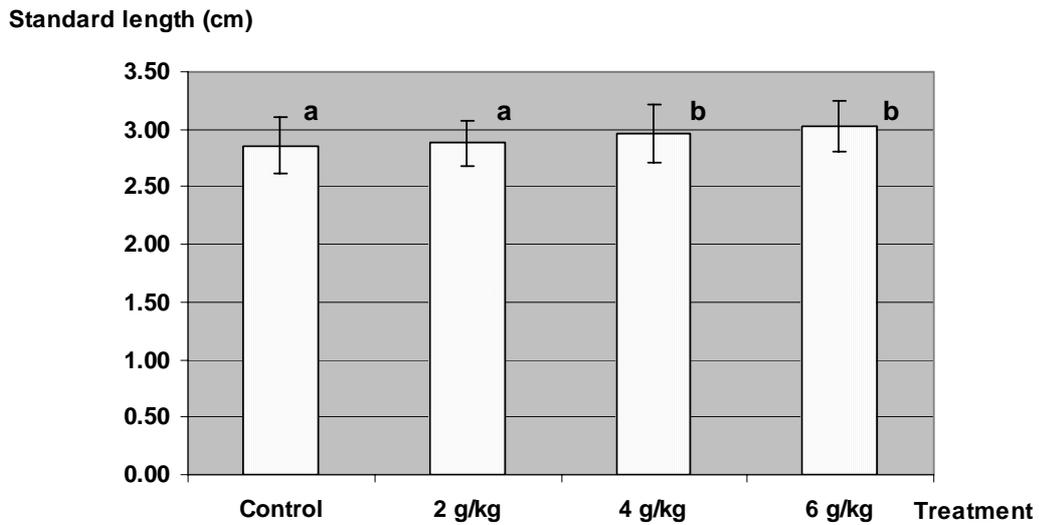


Figure 2. Effect of different concentrations of mannan-oligosaccharide on standard length (Mean \pm S.D.) of Nile tilapia fry

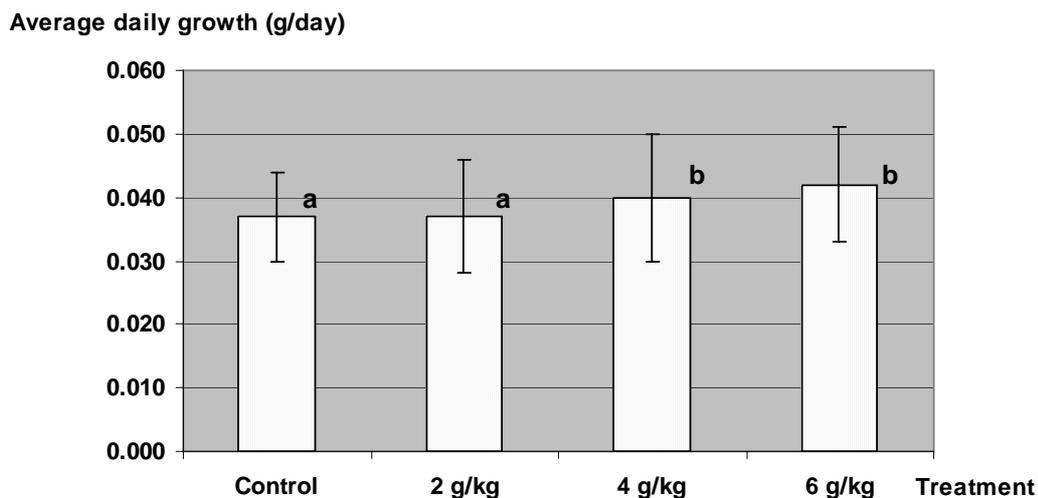


Figure 3. Effect of different concentrations of mannan-oligosaccharide on average daily growth (Mean \pm S.D.) of Nile tilapia fry

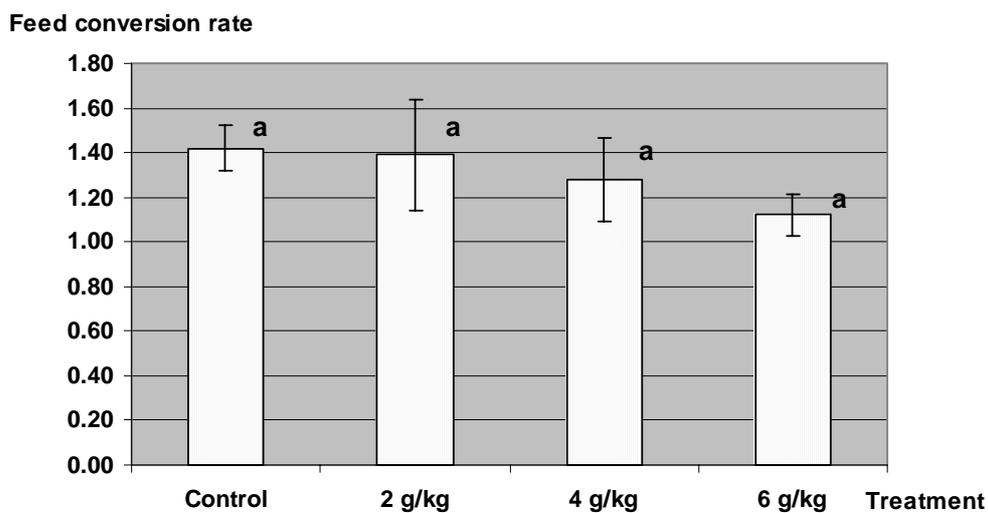


Figure 4. Effect of different concentrations of mannan-oligosaccharide on feed conversion rate (Mean \pm S.D.) of Nile tilapia fry

Survival rates were not significantly different ($P>0.05$) after 21 day trial ranging from 89.67-96.33% (Table 2 and Fig 5)

Table 2. Effect of different concentrations of mannan-oligosaccharide on survival rate (%) of Nile tilapia fry after an experimental period of 21 days (Mean \pm S.D.)

Treatment	Survival rate (%)
Control	89.67 \pm 9.07
2 g/kg	94.00 \pm 10.39
4 g/kg	95.67 \pm 3.21
6 g/kg	96.33 \pm 6.35

Note: No significant difference was detected ($P>0.05$)

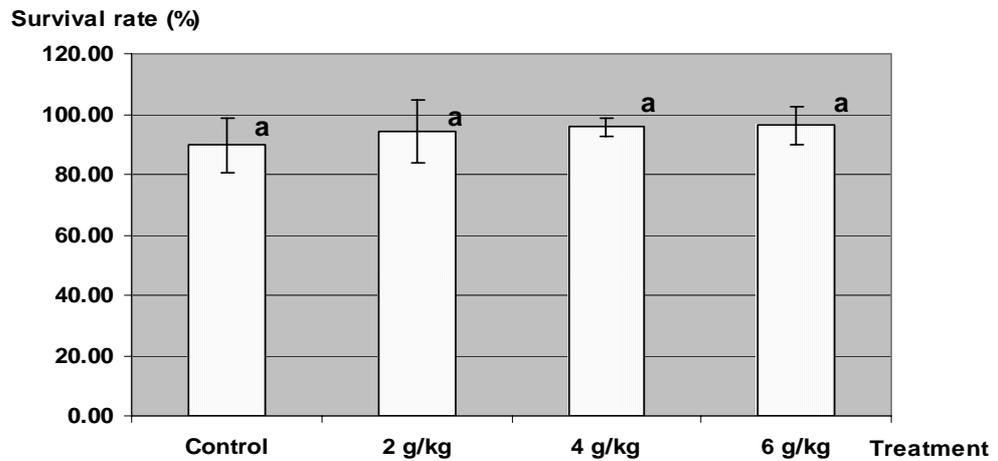


Figure 5. Effect of different concentrations of mannan-oligosaccharide on survival rate (%) (Mean \pm S.D.) of Nile tilapia fry

Resistance against *Streptococcus agalactiae*

It was clearly shown from this experiment that MOS could enhance the immunity of Nile Tilapia fry. All experimental groups fed with MOS had significantly lowered mortality rate than the control one after challenged with virulent *S. agalactiae* ($P<0.05$). Tilapia that were fed with 4 and 6 g/kg MOS did not have any mortality while there was 3.33% mortality in 2 g/kg MOS group and 43.33% of control fish died (Table 3 and Fig 6)

Table 3. Effect of different concentrations of mannan-oligosaccharide on disease resistance against *Streptococcus agalactiae* (Mean \pm S.D.) of Nile tilapia fry

Treatment	Mean percent mortality (\pm S.D.)
Control	43.33 \pm 20.82 ^a
2 g/kg	3.33 \pm 5.77 ^b
4 g/kg	0.00 \pm 0.00 ^b
6 g/kg	0.00 \pm 0.00 ^b

Note: Significant difference was indicated by different superscript letter within the same column at $P=0.05$

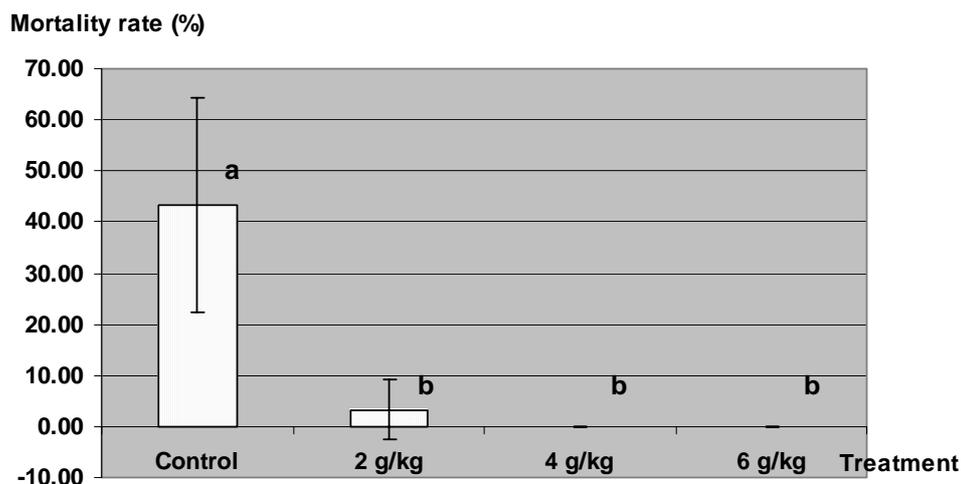


Figure 6. Effect of different concentrations of mannan-oligosaccharide on disease resistance against *Streptococcus agalactiae* (Mean \pm S.D.) of Nile tilapia fry

DISCUSSION AND CONCLUSION

This study clearly indicated the benefit of mannan-oligosaccharide for tilapia fry. With 4 and 6 g/kg MOS in basal diet during 21 day of sex-reversal process, tilapia had significantly better growth rate in term of weight, length and average daily growth. The study on MOS with other freshwater species such as European catfish (*Silurus glanis*) juveniles (Bogut *et al.*, 2006) have shown similar improvement in growth from 22 g to 76 g in the control group and 83 g in the MOS group, a 9.7% higher average body weight ($P < 0.01$). The mortality decreased from 28.33 % to 16.67% ($P < 0.01$). Juvenile carp reared in tanks at the University of Osijek, Croatia also showed similar improvement in weight gain (Culjak *et al.*, 2006). The diet used in these trials was 39.91% crude protein and 4.51% lipid and in the MOS treated groups with an inclusion rate of 0.6%. The fish grew from an average weight of 5.28 g to 31.23 g in the control and 38.73 g in the MOS treated group, a 24% higher weight gain ($P < 0.01$). The FCR significantly improved in the MOS treated groups, decreasing from 2.06 to 1.60 ($P < 0.05$) and the mortality also decreased from 50.0% to 16.7% ($P < 0.01$). In rainbow trout trials (Staykov *et al.*, 2005 a & b) with a 0.2% MOS inclusion rate in commercial feed resulted in significantly increased average weight of 13.7% ($P < 0.001$) in fish grown from 30 g to just under 100 g. Mortalities and FCR were significantly lower in the MOS treated groups. The FCR decreased from 0.91 in the control group to 0.83 in the MOS treated groups ($P < 0.05$). Mortalities decreased from 1.68% in the control groups to 0.58% in the MOS treated groups ($P < 0.001$). At the University of Las Palmas of Gran Canaria in Spain, 35 g sea bass (*Dicentrarchus*

labrax) juveniles showed improved specific growth rate (SGR) and relative growth which increased significantly by about 10% ($P < 0.05$) after fed with MOS at 0.2 and 0.4% inclusion rates (Torrecillas and Izquierdo, 2006). In the MOS treated sea bass, a higher feed intake was observed when compared to the control, however at the end of the experimental period all the groups had similar FCR.

In this present study, survival rate and feed conversion ratio of control and all MOS-treated Nile tilapia (*O. niloticus*) fry were not significantly different after the 21-day trial. This might be related to the length of the trial. However, all concentrations of MOS could significantly enhance the resistance against pathogenic *Streptococcus agalactiae*. In common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss*) that were fed with MOS, had increase bactericidal activity, antibody titres, lysozyme concentration (Lie, 1985), alternative pathway of complement activity (APCA) (Sotirov, 1986) and classical pathway of complement activity (CPCA) (Stelzar and Stein, 1971) when compared with the control. Similar as 0.2% and 0.4% of MOS in European sea bass (*D. labrax*) trial (Torrecillas *et al.*, 2007) which could activate immune system when challenge test with pathogenic *Vibrio alginolyticus*. Further experiments must be conducted to clarify the action mechanisms of MOS with immune system, as well as the optimum feeding period and dose for low capital production.

The results from this study clearly showed the benefits of mannan-oligosaccharide (MOS) as feed supplement on growth and disease resistance against pathogenic bacteria, *Streptococcus agalactiae* during the 21-day nursing period of Nile tilapia fry. On-going research is being conducted to investigate the benefits of MOS on immune functions of Nile tilapia.

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REFERENCES

1. Bogut, I., Z. Milakovic, J. Pavlicevic and D. Petrovic. 2006. Effect of Bio-Mos on performance and health of European catfish, *Sillirus glanis*. Proceedings of Alltech's 22nd Annual Symposium April 23-26, 2006. Lexington, KY, USA.
2. Boonaom, C. 2007. Experimental Design. Dept of Statistic, Faculty of Science, Kasetsart University, Bangkok, Thailand.

3. Culjak, V., I. Bogut, E. Has-Schon, Z. Milakovic and K. Canecki. 2006. Effect of Bio-Mos on performance and health of juvenile carp. Proceedings of Alltech's 22nd Annual Symposium April 23-26, 2006. Lexington, KY, USA.
4. Darinya, J., N. Areechon and P. Srisapoom. 2007. Study on identification and antimicrobial sensitivity test of *Streptococcus* spp. isolated from Nile tilapia (*Oreochromis niloticus*). Proceeding of 44th Kasetsart University Annual Conference 30 Jan – 2 Feb, 2006. Bangkok, Thailand.
5. Lie, O. 1985. Improved agar plate assays of bovine lysozyme and haemolytic complement activity, pp. 1-12. *In* Markers of Resistance to Infection in Dairy Cattle. Dissertation, Oslo, Norway, National Veterinary Institute.
6. Sotirov, L. K. 1986. Method for determination of the alternative pathway of complement activation in some animals and man, pp. 1-10. *In* Fourth Scientific Conference of Agriculture. StaraZagora.
7. Staykov, Y., S. Denev and P. Spring. 2005a. The effects of mannan oligosaccharide (Bio-Mos) on the growth rate and immune function of rainbow trout (*Salmo gairdneri* irideus G.) growth in net cages, pp. 427-432. *In* B. Howell and R. Flos (eds.). Lessons from the past to optimise the future. European Aquaculture Society, Special Publication No.35. June 2005.
8. Staykov, Y., S. Denev and P. Spring. 2005b. The effects of mannan oligosaccharide (Bio-Mos) on the growth rate and immune function of rainbow trout (*Salmo gairdneri* irideus G.) growth in net cages, pp. 429-430. *In* B. Howell and R. Flos (eds.). Lessons from the past to optimise the future. European Aquaculture Society, Special Publication No.35. June 2005.
9. Stelzar, A. and G. Stein. 1971. Moglichkikten zur hamolytishchen Aktivitatsmessung von Gesamtkomplement Theoretische Grundlangen und methodische Hinweise. Wissenschaftliche Zeitschrift der Friedrich Schiler Universitat Uena. Mathematisch Naturwissenschaftliche Reihe 20, Jg H6: 933-941.
10. Torrecillas, S. and M. S. Izquierdo. 2006. The effect of Bio-Mos on European sea bass (*Dicentrarchus labrax*) juveniles: growth performance and feed utilization. Proceedings of Alltech's 22nd Annual Symposium April 23-26, 2006. Lexington, KY, USA.
11. Torrecillas S., A. Makol, M. Jcaballero, D. Montero, L. Robaina, F. Real, J. Sweetman, L. Tort and M. S. Izquierdo. 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish & Shellfish Immunol. 23: 969-981.