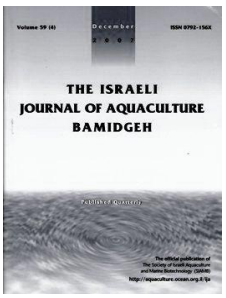
The Israeli Journal of Aquaculture - Bamidgeh, IIC:63.2011.619, 8 pages



Effect of Mannan Oligosaccharide on Growth, Body Composition, and Antioxidant Enzyme Activity of Tilapia (*Oreochromis niloticus*)

Arzu Özlüer-Hunt[1]*, Mehmet Berköz[2], Ferbal Özkan[1], Serap Yalin[3], Zeynep Erçen[4], Erhan Erdoğan[4], Suna Gül Gündüz[1]

[1] *Department of Aquaculture, Faculty of Fisheries, Mersin University, 33160 Mersin, Turkey*

[2] *Department of Pharmaceutical Technology, Faculty of Pharmacy, Mersin University, 33160 Mersin, Turkey*

[3] *Department of Biochemistry, Faculty of Pharmacy Mersin University, 33160 Mersin, Turkey*

[4] *Department of Aquaculture, Faculty of Fisheries, Cukurova University, 01330 Adana, Turkey*

(Received 9.8.10, Accepted 14.12.10)

Key words: mannan oligosaccharides, growth, tilapia, yeast, antioxidant, enzyme

Abstract

The effects of three inclusion levels of mannan oligosaccharides (MOS) derived from the outer cell wall of a select strain of *Saccharomyces cerevisiae* (Bio-Mos, Alltech Inc, USA) on growth, feed utilization, body composition, and antioxidant enzyme activity of tilapia (*Oreochromis niloticus*) were determined. Specimens (12 g) were fed a commercial diet supplemented with 0‰, 2.5‰, 3.5‰, or 4.5‰ dietary MOS for 60 days. Food conversion rates (FCR), specific growth rates (SGR), and the biochemical composition of muscle tissue were determined. Growth was greatest, protein was highest, and lipid was lowest in fish fed the 2.5‰ MOS diet. The FCR was significantly better in all MOS-treated groups than in the unsupplemented control. At the end of the study, antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in liver and muscle tissue was generally higher in the supplemented diets than in the control and significantly higher in the 4.5‰ treatment. Oxidative damage due to lipid peroxidation (LPO) was assessed by formation of malondialdehyde (MDA) and protein carbonyl (PC), both of which were significantly lower in liver tissue in all MOS-supplemented diets than in the control.

* Corresponding author. Tel.: +90-324-3412815-2101, fax: 90-324-3413025, e-mail: arzuozluer@hotmail.com, ahunt@mersin.edu.tr

Introduction

For years, the most common method of dealing with bacterial infections in aquaculture was the administration of antibiotics. However, serious adverse effects of these drugs include accumulation in fish tissues, immunosuppression, development of antibiotic-resistant bacteria, and destruction of environmental microbial flora. Further, antibiotics and vaccines are expensive or unavailable in many areas. These factors have generated interest in the use of probiotics and prebiotics (Ganguly et al., 2010), many of which are yeast-based products incorporated into the diet as whole cells or cellular derivatives.

Mannan oligosaccharide (MOS) is derived from the outer cell wall of the yeast, *Saccharomyces cerevisiae*. Two major polysaccharides constitute up to 90% of the dry weight of the cell wall: α-D-mannan and β-D-glucan. Modulation of mucosal immunity by the binding of these two polysaccharides to specific receptors of immune cells benefits animal health and increases disease resistance. Both polysaccharides have remarkable properties that interact with the immune system of the host by enhancing antioxidant activities (Križková et al., 2001). In addition, MOS can enhance growth and improve gut function and health by increasing villi height, uniformity, and integrity in fish (Staykov et al., 2007; Torrecillas et al., 2007; Dimitroglou et al., 2009).

Antioxidant enzymes are natural defenses that can be enhanced under stressful situations and used as indicators of oxidative stress (Livingstone, 2001). *In vitro* and *in vivo* studies demonstrate the capacity of fish to generate reactive oxygen species (ROS) by xenobiotics, physical conditions, and/or diet. Measurements of antioxidative enzyme activity in fish are used to evaluate oxidative damage caused by environmental factors in aquatic ecosystems (Zhang et al., 2004). In all organisms, the main antioxidative enzymes for detoxification of ROS are glutathione peroxidase (GSH-Px; EC 1.11.1.9), superoxide dismutase (SOD; EC 1.15.1.1), and catalase (CAT; EC 1.11.1.6).

GSH-Px is the most important peroxidase for detoxification of hydroperoxides. CAT primarily occurs in peroxisomes where it detoxifies H_2O_2 to O_2 and water. The key role of the SOD enzyme is to dismutase the superoxide anion in hydrogen peroxide, which is then used as a substrate by CAT and GPx enzymes (Elia et al., 2011). Malondialdehyde (MDA) is the main oxidation product of peroxidized polyunsaturated fatty acids (Elia et al. 2002). Protein carbonyls (PC) are normally used as a biomarker for protein damage caused by oxidized amino acid residues in stress conditions (Barelli et al., 2008).

If the antioxidants in a body are unable to overwhelm the free radicals, free radical activity can lead to cell damage known as oxidative stress. Antioxidants can protect organisms from free radicals and ROS effects and slow down the progress of many chronic diseases as well as LPO (Kim et al., 2009). The purpose of this research was to determine the effects of dietary MOS on the antioxidant response of tilapia and the optimum dietary level for maximum growth. Therefore, in the present study we investigate the effects of MOS supplementation on growth performance, muscle composition, activity of GSH-Px, SOD, and CAT, and levels of MDA and PC in the liver and muscle of tilapia (*Oreochromis niloticus*).

Materials and Methods

Fish and experimental systems. The experiment was carried out in the outdoor installations of the Fresh Water Unit of the Faculty of Fisheries in Cukurova University, Adana, Turkey. The experimental system consisted of 12 experimental cages (1 × 1 × 1.25 m) located inside a large concrete pond. Water depth was maintained at 1 m and water was continuously added throughout the experiment. Fresh water was supplied by pipe from the Seyhan Dam drainage system. Cages were thoroughly cleaned every two weeks after fish sampling. Dissolved oxygen was measured every other day using YSI model 58 oxygen meters (Yellow Springs Instrument Company, Yellow Springs, OH) and pH was monitored once a week with an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH). Total NO_3, NO_2, and N-NH_4 were monitored weekly using a Merck-Spectroquant® Nova 60 A. Water temperature was recorded daily at 13:00 with a mercury thermometer suspended at a depth of 30 cm.

Three hundred tilapia, *Oreochromis niloticus* (12 g), were obtained from the Fresh Water Fish Unit in Cukurova University, Adana, Turkey, and stocked into 1000-l cement ponds for two weeks for acclimation. During this period fish were fed a commercial diet (2 mm Bio Aqua, Camli Yem, İzmir, Turkey) at 3% body weight daily. After the initial acclimatization, the fish were weighed, randomly distributed into experimental cages at 10 fish/cage (120 fish in all), and further acclimatized for 2 days without feed.

During the experiment fish were fed either a commercial basal diet (Table 1) or the basal diet supplemented with 2.5‰, 3.5‰ or 4.5‰ organic yeast (MOS; Bio-Mos®, Alltech Inc. USA). The commercial diet was crushed, mixed with sufficient water and Bio-MOS (2.5, 3.5 and 4.5 g MOS/kg), and made again into pellets. The pellets were allowed to dry and were stored at 4°C until use. The control diet was similarly treated, but without the MOS inclusion.

Table 1. Composition of basal diet (dry matter basis).

Nutrient	*Content (%)*
Moisture	12.92
Crude protein	45.39
Crude fat	12.46
Dry matter	87.08
Ash	8.28

Vitamin/mineral supplement per kg feed: folic acid 5 mg, pantothenic acid 62 mg, coline 560 mg, vitamin A 15,000 IU, vitamin B1 13 mg, vitamin B12 0.50 mg, vitamin B2 25 mg, vitamin B6 12 mg, vitamin C 600 mg, vitamin D3 2500 IU, vitamin E, 62 mg, vitamin K3 6.50 mg, niacin 125 mg, inositol 10 mg, Cu 5 mg, Co 0.26 mg, Fe 63 mg, I 1.65 mg, Mn 25 mg, Se 0.13 mg, Zn 75 mg

Fish in three replicate cages were fed calculated quantities of feed in two equal portions (08:00, 16:00) for 60 days. Fish from each cage were weighed biweekly and food rations were adjusted to 3% body weight. To reduce stress, no feed was given on sampling days.

Proximate analysis. For proximate analysis, six fresh fish from each cage were ground together. Fillets were analyzed for moisture, protein, lipid, and ash using standard methods (AOAC, 1996): moisture by heating samples at 60°C to a constant weight, protein by estimating Kjeldahl nitrogen (× 6.25) in an automated distillation unit, lipid by chloroform/methanol extraction, and ash by incineration in a muffle furnace at 550°C for 18 h. Analyses were done in triplicate.

Determination of antioxidant enzymes. For analysis of liver and muscle antioxidant enzymes, and lipid peroxidation, 24 fish (six fish/group) were sacrificed. Total GSH-Px activity was assayed by the Jocely (1970) method using H_2O_2 and NADPH as substrates. Conversion of nicotinamide adenine dinucleotide phosphate (NADPH) to nicotinamide adenine dinucleotide phosphate (NADP) was followed by recording the changes in absorption intensity at 340 nm; one unit was expressed as one mole of NADPH consumed per minute using a molar extinction coefficient of 6.22×10^6. SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2 generated by the xanthine/xanthine oxidase system (Sun et al., 1988). One unit of SOD activity was defined as the amount of protein that caused 50% inhibition of the NBT reduction rate. CAT activity was determined according to the method of Aebi (1974). Enzymatic decomposition of H_2O_2 was directly followed by a decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. Enzyme activity is given in U/mg protein.

Detection of lipid peroxidation and protein oxidation (protein carbonyls). MDA levels on homogenized tissues (an index of lipid peroxidation) were determined by thiobarbituric acid reaction using the method of Yagi (1998). PC levels on homogenized tissues were determined by 2,4 dinitrophenyl-hydrazine (DNPH) reaction using the method of Levine et al. (1990). Tissue protein contents were determined according to the method developed by Lowry et al. (1951) using bovine serum albumin as the standard.

Statistical analysis. All results were analyzed by one-way analysis of variance (ANOVA). When ANOVA identified differences between groups, multiple comparisons among means were made with Duncan's new multiple range test. Differences were considered significant at $p<0.05$.

Results

Average water quality parameters were temperature 27.5±0.8°C, dissolved oxygen 7.1±0.07 mg/l, pH 8.2±0.19, NO_3 0.4±0.1 mg/l; NO_2 0.06±05 mg/l, and NH_4 0.08±0.5 mg/l. There were no obvious effects of MOS on water quality in any treatment group. No mortality occurred during the trial. After 60 days, the final weight and specific growth rate were significantly higher in the 2.5‰ MOS-supplemented than in the control (Table 2). The feed conversion ratio was significantly better in all MOS-treated groups than in the control and the protein efficiency ratio was significantly best in the 2.5‰ MOS group. Muscle protein content was highest in the 2.5‰ MOS group. In general, GSH-Px, SOD, and CAT activity in the liver and muscle increased with the dietary inclusion of MOS while MDA and PC levels significantly decreased in the liver of fish fed MOS-supplemented groups (Table 3).

Table 2. Growth performance, feed utilization, and final muscle composition in tilapia (*Oreochromis niloticus*) fed diets containing different levels of mannan oligosaccharides (MOS; means±SEM; n = 3) for 60 days.

	Dietary treatment (MOS content)			
	Control	*2.5‰*	*3.5‰*	*4.5‰*
Initial wt (g)	12.19±0.06	12.18±0.03	12.21±0.01	12.25±0.06
Final wt (g)	59.24±0.51^a	68.57±0.81^b	58.32±0.67^a	55.25±0.51^c
Live wt gain (%)	387.23±4.38^a	463.07±6.14^b	377.63±4.94^a	356.46±6.46^c
Dry wt gain (g)	0.78±0.08^a	0.94±0.01^b	0.76±0.01^a	0.72±0.08^c
SGR [1]	2.64±0.01^a	2.85±0.01^b	2.60±0.01^a	2.53±0.02^c
FCR[2]	1.79±0.05^a	1.56±0.03^b	1.62±0.05^b	1.54±0.01^b
PER[3]	1.02±0.08^a	1.23±0.03^b	1.00±0.01^a	0.90±0.01^a
Final muscle proximal composition (g 100/g dry weight; n = 6)				
Moisture	77.91±0.13	77.63±0.11	78.40±0.35	77.74±0.28
Crude protein	22.73±0.44^a	23.98±0.20^b	22.14±0.03^a	22.82±0.13^a
Crude lipid	2.90±0.03^a	2.47±0.08^b	2.51±0.12^b	2.18±0.16^b
Ash	1.87±0.01	1.89±0.06	1.92±0.02	1.85±0.01

Different superscripts within a line denote significant difference (p>0.05).
[1] Specific growth rate = 100($\log_e$ avg final wt - $\log_e$ avg initial wt)/no. days
[2] Feed conversion rate = feed intake/wet wt gain
[3] Protein efficiency ratio = wt gain/protein intake

Table 3. Activity of antioxidant enzymes (U/mg) and levels of oxidation products (nmol / mg protein) in muscle and liver tissues of *Oreochromis niloticus* fed diets with different levels of mannan oligosaccharides (MOS) for 60 days (means±SEM; n = 6).

	Dietary treatment (MOS content)			
	Control	*2.5‰*	*3.5‰*	*4.5‰*
Muscle				
GSH-Px	8.44±1.31^a	10.80±1.33^a	11.15±1.37^a	13.92±1.73^b
SOD	86.67±4.37	91.13±5.68	93.58±4.54	92.29±5.78
CAT	61.53±2.13	63.94±2.24	65.28±2.26	73.74±2.62
MDA	88.92±5.23	90.18±4.98	89.38±6.92	92.76±5.45
PC	1.33±0.06	1.22±0.10	1.28±0.11	1.30±0.15
Liver				
GSH-Px	13.22±1.65^b	15.71±1.23^b	22.02±1.75^c	22.56±1.76^c
SOD	108.12±4.76^b	99.85±3.38^b	128.82±4.03^c	141.2±5.80^c
CAT	72.33±3.59^b	76.36±4.01^b	82.47±4.72^c	88.66±4.26^c
MDA	137.51±8.99^b	96.82±5.34^c	73.16±5.07^d	51.96±5.01^a
PC	1.55±0.10^b	1.47±0.11^b	0.85±0.08^c	0.61±0.06^c

Different superscripts within a line denote significant difference (p>0.05).
GSH-Px = glutathione peroxidase, SOD = superoxide dismutase, CAT = catalase, MDA = malondialdehyde, PC = protein carbonyl

Discussion

The inclusion of dietary MOS significantly affected growth. The group fed the 2.5‰ MOS diet grew significantly best while growth of the 4.5‰ MOS group was lower growth than in the control. Earlier studies found contradictory effects of MOS on growth performance in aquatic species. Dietary supplementation of prebiotics was not recommended for hybrid striped bass (Li and Gatlin, 2005), common carp (Staykov et al., 2005), European sea bass (Torrecillas et al., 2007), and gilthead sea bream (Dimitroglou et al., 2010). Likewise, MOS supplementation did not improve growth performance in hybrid tilapia (Genc et al., 2007), cobia larvae (Salze et al., 2008), or channel catfish (Peterson et al., 2010). These discrepancies regarding the effect of MOS on growth might be dependent upon the type of yeast, fish species, or feeding period (Welker et al., 2007). In the current study, we followed the manufacturer's recommended dietary concentrations for aquatic species, but the effective concentration of dietary MOS can vary depending on feeding period, fish species, age, nutritional status, and physiological condition.

Dietary MOS increases the absorptive surface area and microvilli density and length (Staykov et al., 2007; Salze et al., 2008; Sang and Fotedar, 2010). Improvement of gut morphology is likely to benefit not only feed utilization but also maintenance of an intact, healthy mucosal epithelium that can help prevent infection by opportunistic indigenous bacteria. Gut bacteria is a combination of naturally-occurring bacteria (opportunistic bacteria) and beneficial bacteria which result from the use of prebiotics (Muthu Ramakrishnan et al., 2008). Higher numbers of aerobic microbiota in the gut may increase host survival in suboptimal dietary conditions by improving digestion efficiency and providing digestive enzymes or vitamins (Sang and Fotedar, 2010). Thus, the digestive, absorptive, and assimilated processes of the food intake could have been improved by including MOS in the diets, resulting in the higher growth rate.

We did not measure the density of fish microvilli but the FCR was significantly lower in all MOS-supplemented groups than in the control. Similar results were obtained in rainbow trout in which a significant increase in average weight (13.7%; $p<0.001$) and significant drops in mortality and FCR ($p<005$) were obtained in MOS-treated groups (Staykov et al., 2005). In our study, supplementation at 2.5‰ MOS significantly improved the PER, contributing to the optimization of protein for growth. However, although the FCR improved when MOS was included at 4.5‰, growth performance was lower than in the control. Thus, when included at an acceptable dietary level, MOS can lower FCR in cultured fish, leading to increased weight gain.

MOS supplementation at 2.5‰ significantly improved the protein content and decreased the fat content in the muscle tissue when compared to the control. Similar results were obtained in European sea bass fed MOS-supplemented diets and histological features of the liver showed lower lipid vacuolization and regular-shaped morphology of hepatocytes around sinusoidal spaces denoting better utilization of dietary nutrients (Torrecillas et al., 2007). Thus, we conclude that MOS significantly reduces muscle lipid in the edible component of fish, providing a leaner and potentially healthier product.

When compared to the control, GSH-Px, CAT, and SOD activity increased and MDA and PC concentrations significantly decreased in the liver of fish fed the MOS-supplemented diets. In the muscle, GSH-Px and CAT activity slightly increased in fish fed the supplemented diets, especially at the 4.5‰ MOS inclusion level. MDA and PC were not altered in muscle tissue when compared to the control. The cause of the differences between tissues could be their different rates of free radical generation and antioxidant potential. Of all organs, the liver has the highest level of antioxidant enzymes (Lin and Lin, 1997). The increases in enzymatic GSH-Px, SOD, and CAT antioxidants in the livers show activation of antioxidant protection, which is needed even in healthy fish during the normal feeding cycle. Many feed supplements, herbs, bio-stimulating, and immunostimulating compounds stimulate the antioxidant systems of animals. Addition of Bio-Mos stimulated SOD, CAT, and GSH-Px activity in sows (Czech et al., 2009). On the other hand, while SOD activity significantly rose in *Sparus aurata* fed a yeast diet, CAT activity was not affected (Reyes-Becerril et al., 2008). But, similar to our results, SOD and CAT activity significantly increased in shrimp (Wang et al., 2009) and in the liver of

carp fingerlings fed diets supplemented with organic selenium yeast (Jovanovic et al., 1997). While SOD activity may indicate increased metabolic activity, yeast is a potential source of microelements such as copper, iron, and zinc, that are crucial for the synthesis of SOD and CAT (Jovanovic et al., 1997). Further, one component of the yeast cell wall is β-D-glucan which has powerful antioxidant attributes with heightened free-radical scavenging activity that enables the immune system to fight fungi, bacteria, viruses, parasites, and radiation (Campa-Córdova et al., 2002; Kim et al., 2009). The health, growth, and general performance of farmed shrimp and fish may be improved by the use of β-glucans (Ganguly et al., 2010). β-glucan modulates antioxidant enzyme activity and inhibits lipid peroxidation in some animals (Kim et al., 2009). Our results suggest that an increase in antioxidants is possibly related to MOS supplementation.

In conclusion, MOS supplementation in tilapia diets improves growth performance and enhances antioxidant enzyme activity. The use of functional feed additives such as MOS is increasingly important as consumers increasingly demand eco-friendly production.

Acknowledgements

The authors would like to thank Alltech Inc., USA, for supplying Bio-Mos® free of charge for use in this experiment and Mr. Robert J. Hunt for kindly revising the English. Thanks are also due to all involved workers.

References

Aebi H., 1974. Methods of enzymatic analysis. pp. 671-684. In: H.U. Bergmayer (ed.). *Catalase.* Academic Press, New York.

AOAC, 1996. *Official Methods of Analyses of the Association of Official Analytical Chemists*. Assoc. Official Analytical Chemists, Arlington, VA, USA.

Barelli S., Canellini G., Thadikkaran L., Crettaz D., Quadroni M., Rossier J.S., Tissot J.D. and N. Lion, 2008. Oxidation of proteins: basic principles and perspectives for blood proteomics. *Proteomics Clin. Appl*., 2:142-157.

Campa-Córdova A.I., Hernández-Saavedra N.Y. and F. Ascencio, 2002. Superoxide dismutase as modulator of immune function in American white shrimp (*Litopenaeus vannamei*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmicol.,* 133:557-565.

Czech A., Mokrzycka A., Grela E.R. and Z. Pejsak, 2009. Influence of mannanoligosaccharides additive to sows diets on blood parameters of sows and their piglets. *Bull. Vet. Inst. Pulawy,* 53:89-95.

Dimitroglou A., Merrifield D.L., Moate R., Davies S.J., Spring P., Sweetman J. and G. Bradley, 2009. Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Anim. Sci*., 87:3226-3234.

Dimitroglou A., Merrifield D.L., Spring P., Sweetman J., Moate R. and S.J. Davies, 2010. Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture,* 300:182-188.

Elia A.C., Waller W.T. and S.T.J. Norton, 2002. Biochemical responses of bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. *Bull. Environ. Contam. Toxicol*., 68:809-816.

Elia A.C., Prearo M., Pacini N., Dörr A.J.M. and M.C. Abete, 2011. Effects of selenium diets on growth, accumulation and antioxidant response in juvenile carp. *Ecotoxicol. Environ*. *Saf.,* 74:166-173.

Ganguly S., Paul I. and S.K. Mukhopadhayay, 2010. Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: a review, *Isr. J. Aquacult. - Bamidgeh*, 62(3):130-138.
http://www.siamb.org.il/Content_siamb/editor/62_3_1_Mukhopadhyay_abs.pdf

Genc M.A., Yilmaz E., Genc E. 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*). *Isr. J. Aquacult. - Bamidgeh*, 59(1):10-16. http://www.siamb.org.il/uploads/59_1_Genc%20abs.pdf

Jocely P.C., 1970**.** The enzymic oxidation of glutathione in rat liver homogenates. *Biochem. J.*, 117:947-949.
Jovanovic A., Grubor-Lajsic G., Djukic N., Gardinovacki G., Matic A. and M. Spasic, 1997. The effects of selenium on antioxidant system in erythrocytes and liver of the carp (*Cyprinus carpio* L.). *Crit. Rev. Food Sci. Nutr.,* 37:443-448.
Kim Y.S., Ke F. and Q.Y. Zhang, 2009. Effect of β-glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. *Fish Shellfish Immunol.,* 27:336-340.
Križková L., Ďuračková Z., Šandula J., Sasinková V. and J. Krajčovič, 2001. Antioxidative and antimutagenic activity of yeast cell wall mannans *in vitro*. *Mutat. Res*. 497:213-222.
Levine R.L., Garland D., Oliver C.N., Amici A., Climent I., Lenz A.G., Ahn B.W., Shaltiel S. and E.R. Stadtman, 1990. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol*., 186:464-478.
Li P. and D.M. Gatlin III, 2005. Evaluation of the prebiotic GroBiotic®-A and brewers yeast as dietary supplements for subadult hybrid striped bass (*Morone chrysops* × *M. saxatilis*) challenged *in situ* with *Mycobacterium marinum*. *Aquaculture,* 248:197-205.
Lin C.T. and M.T. Lin, 1997. The distribution and properties of superoxide dismutase in different tilapia tissues. *J. Chin. Agric. Chem. Soc*., 35:107-111.
Livingstone D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull.,* 42:656-666.
Lowry O.H., Rosebrough N.J., Farr A.L. and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193:265-275.
Muthu Ramakrishnan C., Haniffa M.A., Manohar M., Dhanaraj M., Jesu Arockiaraj, A., Seetharaman S. and S.V. Arunsingh, 2008. Effects of probiotics and spirulina on survival and growth of juvenile common carp (*Cyprinus carpio*). *Isr. J. Aquacult. - Bamidgeh*, 60(2):128-133.
http://www.siamb.org.il/uploads/60_2_arokaiaraj%20abs.pdf
Peterson B.C., Bramble T.C. and B.C. Manning, 2010. Effects of Bio-Mos® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri. J. World Aquacult. Soc.,* 41(1):149-155.
Reyes-Becerril M., Tovar-Ramírez D., Ascencio-Valle F., Civera-Cerecedo R., Gracia-López V. and V. Barbosa-Solomieu, 2008. Effects of dietary live yeast *Debaryomyces hansenii* on the immune and antioxidant system in juvenile leopard grouper *Mycteroperca r*osacea exposed to stress. *Aquaculture*, 280:39-44.
Salze G., McLean E., Schwarz M.H. and S.R. Craig, 2008. Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. *Aquaculture,* 274:148-152.
Sang H.M. and R. Fotedar, 2010. Effects of mannan oligosaccharide dietary supplementation on performances of the tropical spiny lobsters juvenile (*Panulirus ornatus*, Fabricius 1798). *Fish Shellfish Immunol.*, 28:483-489.
Staykov Y., Denev S. and P. Spring, 2005. Influence of dietary mannan oligosaccharides (Bio-Mos) on growth rate and immune function of common carp (*Cyprinus carpio* L.). pp. 431-432. In: B. Howal, R. Flos (eds.)*. Lessons From the Past to Optimize the Future*. Eur. Aquacult. Soc. Special Publ. 35. EAS, Oostende, Belgium.
Staykov Y., Spring P., Denev S. and J. Sweetman, 2007. Effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Int*., 15:153-161.
Sun Y., Oberley L.W. and L. Ying, 1988. A simple method for clinical assay of superoxide dismutase. *Clin. Chem.*, 34:497-500.
Torrecillas S., Makol A., Caballero M.J., Montero D., Robaina L., Real F., Sweetman J., Tort T. 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.
Yagi K., 1998. Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. *Methods Mol. Biol.*, 108:107-110.

Wang H.W., Cai D.B., Xiao G.H., Zhao C.L., Wang Z.H., Xu H.M. and Y.Q. Guan, 2009. Effects of selenium on the activity of antioxidant enzymes in the shrimp, *Neocaridina heteropoda. Isr. J. Aquacult. - Bamidgeh,* 61(4):322-329. http://www.siamb.org.il/Content_siamb/editor/61_4_Wang_abs.pdf

Welker T.L., Lim C., Yıldırım-Aksoy M., Shelby R. and P.H. Klesius, 2007. Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. *J. World Aquacult. Soc.*, 38(1):24-35.

Zhang J., Shen H., Wang X., Wu J. and Y. Xue, 2004. Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus. Chemosphere*, 55:167-174.