The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

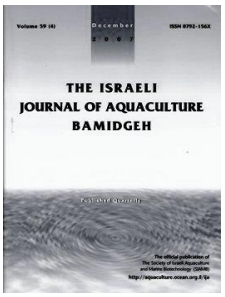
This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawai'i at Mānoa Library
&
University of Hawai'i at Mānoa Aquaculture Program
in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il



Effects of Prebiotic Mannanoligosaccharides (MOS) on Histology and Biochemical Blood Parameters of Gilthead Seabream, *Sparus aurata*

Nejdet Gültepe[1]*, Osman Sabri Kesbiç[2], Ümit Acar[3], Kutalmış Gökkuş[2], M.İrşad Gültepe, Adem Yavuz Sönmez[4], Soner Bilen[4], Seyit Aydın[1]

[1] Kastamonu University, Engineering and Architecture Faculty, Department of Genetic and Bioengineering, 37200, Kastamonu, Turkey
[2] Kastamonu University, Inebolu Vocational School, Sea and Port Management Program, 37500, Inebolu, Kastamonu, Turkey
[3] Muğla Sıtkı Koçman University, Department of Aquaculture, Faculty of Fisheries, 48000, Kötekli, Muğla, Turkey
[4]Kastamonu University, Fisheries Faculty, Department of Basic Sciences, 37200, Kastamonu, Turkey

(Received 14.1.2014, Accepted 23.3.2014)

Keywords: Mannanoligosaccharides, gilthead seabream, *Sparus aurata,* histology, blood biochemistry, growth performance

Abstract

A 90-day growth trial was conducted to determine the effects of prebiotic mannanoligosaccharides (MOS) on the histology and biochemical blood parameters of the gilthead seabream (*Sparus aurata*). Two experimental diets were formulated to contain MOS (Bio-Mos®) levels of 0 and 2 g/kg. The fish averaging 172.11±13.19 g were reared in six octagonal net cages (763 m^3). Fish were fed twice daily to apparent satiation. Individual body weight, weight gain, survival rate and feed conversion rate FCR were measured during the experiment. The treatments produced significant differences in body weight. and weight. gain ($p<0.050$) but no significant effect on the survival rates and FCR ($P>0.05$). At the end of the experimental period, biochemical blood parameters were analyzed. Blood urea level was significantly affected in the MOS group ($P<0.05$). No remarkable alterations in histological examinations were found. This study highlighted the positive effects of prebiotic MOS on protein efficiency and weight gain of gilthead seabream.

*Corresponding author: ngultepe@kastamonu.edu.tr

Introduction

Biotechnology has contributed to great progress in animal husbandry. In the 1980's, oligosaccharides such as fructo-, galacto-, glycol-, malto-, and mannanoligosaccharide (MOS) began attracting the attention of researchers. In the 1990's, oligosaccharides were tested on some animal species. MOS were very effective additives for boosting the immune system and gastrointestinal microflora in animals (Newman, 1994). Prebiotics and probiotics are now used in aquaculture (Ganguly et al., 2010). MOS are glucomannoproteins derived from the cell walls of yeast. Studies have shown the positive effects of MOS (Genc et al., 2007; Yılmaz et al., 2007; Gültepe et al., 2011; 2012).

Due to lack of standard values in fish, blood chemistry is not examined extensively in aquaculture. However, blood chemistry parameters can provide information on the health status of fish and gauge aspects such as environmental impact, nutritional condition, physiological changes, and presence of disease (Aydın et al., 2000; Coz-Rakovac et al., 2005). Histological examinations of some organs reflect the general physiological condition of fish. MOS has specifically improved gut morphology in some fish species (Sweetman and Davies, 2006).

Gilthead seabream (*Sparus aurata*) is one of the most important fish species in aquaculture in Mediterranean countries. Therefore, the objective of the present study was to examine the effect of a commercial prebiotic MOS feed product Bio-Mos® (Alltech Biotechnology, Lexington, KY, USA) on histology and biochemical parameters of the blood plasma in gilthead seabream.

Materials and Methods

Fish, diet, facilities, and feeding protocol. The experiment was conducted at a commercial gilthead seabream production farm (Kılıç Seafish Aquaculture Import-Export Co.) Bodrum in Muğla, Turkey from September to November 2013 (90 d) using gilthead bream (initial average weight 172.11±13.19 g). At the start of the experiment 60,000±1,260 fish were distributed among six octagonal net cages with an individual volume of 763 m^3. The test diets of 3 mm pelleted feed (Kılıç Feed Factory, Milas, Muğla, Turkey) were supplemented with 0 (control) and 2 g/kg MOS (Table 1). Proximate analyses of the feedstuffs and diets were performed using standard methods (AOAC, 2000). The carbohydrate content of the experimental diets was evaluated (Gouveia and Davies, 2000). Gross energy was determined by adiabatic bomb calorimetry (Parr 6300 adiabatic bomb calorimeter, Parr Instrument Co., Moline, Illinois). Two experimental diets were fed to triplicate groups of fish. During the trial the fish were fed twice daily to apparent satiation. Growth performance of gilthead seabream fed with the diets was evaluated by calculating mean weight gain (WG), feed conversion rate (FCR) and survival (%), where: WG=final weight-initial weight; FCR=feed consumption/WG

Water temperature was virtually identical for the entire experimental period, ranging from 26.4^{0}C to 19.7^{0}C. Salinity, dissolved oxygen, pH, SO_4, PO_4, NO_3, and NO_2 of water were 36±0.5 g/ml, 8.2±0.5 mg/l, 7.7, 0.15 mg/l, 0.19 mg/l, 0.55 mg/l, respectively.

Table 1. Composition of the control and experimental diet for *Sparus aurata*.

Diets	*Control (g/kg)*	*Experimental diet(g/kg)*
Ingredients (g/kg)		
Fish meal	400	400
Soybean meal	220	220
Corn gluten	80	80
Wheat gluten	40	40
Wheat	160	158
Fish oil	79.75	79.75
DL-methionine	2	2
Antioxidant	0.25	0.25
Carboxymethyl cellulose	3	3
Vitamin mix[a]	3	3
Mineral mix[b]	2	2
Bio-Mos®	0	2
Chromic oxide[c]	10	10
Chemical composition		
Dry matter	89.90	90.01
Protein	44.20	44.24
Lipid	12.50	12.51
Ash	8.21	8.20
Carbohydrate	23.49	24.39
Gross energy (kJ)	1.604	1.620

[a]Provided per kg of diet: 4500 IU retinyl acetate (Vit. A), 3600 IU cholecalcipherol (Vit. D), 90 IU DL-a-tocopheryl-acetate (Vit. E), 1.8 mg menadione sodium bisulphate (Vit. K), 0.04 mg cyanocobalamin (Vit. B12), 90 mg ascorbyl polyphosphate (ascorbic acid), 25.2 mg Dbiotin, 1800 mg choline chloride, 1.8 mg folic acid, 18 mg niacin (nicotinic acid), 36 mg pantothenic acid,9 mg pyridoxine, 10.8 mg riboflavin, 1.8 mg thiamin.

[b]Provided per kg of diet: 2.46 mg sodium chloride (NaCl), 0.05 mg ferrous sulphate ($FeSO_4$), 0.02 copper sulphate ($CuSO_4$), 0.07 mg manganese sulphate ($MnSO_4$), 0.008 mg potassium iodide (KI), 0.01 mg zinc sulphate ($ZnSO_4$).

[c]Supplied by SIGMA, St Louis, MO, United States.

Sampling and analytical procedure of blood. At end of the feeding trial, fish were taken from each cage without use of anesthetic, in order to avoid any effects of anesthesia on blood parameters. Fish handling time was less than 1 min per fish. Thus, the total capture time was less than 8 min for each tank. About 4 ml blood was drawn from the caudal vein and immediately transferred to test tubes. The extracted blood was then centrifuged at 4000 rpm for 10 min to separate the serum. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes, and phosphate (PO_4), elemental iron (Fe), sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and chloride (Cl) , and concentration of total protein (TP), glucose (GLC), cholesterol (CHOL), triglyceride (TRIG), creatinine (CREA), direct bilirubin (DBIL), indirect bilirubin (IBIL), urea (URE) and uric acid (URICA) were determined using a Beckman Coulter-LX20 autoanalyzer (Beckman Coulter Inc.).

Histopathological examination. At the end of the feeding trial, ten fish per cage were euthanatized with an overdose of MS-222 (200 mg/l water) for 10 min. for liver collection. Livers and muscles were carefully removed, immediately fixed in a 10% buffered solution of formalin, dehydrated in a graded ethanol series, and embedded in paraffin. Sections were cut at 5 µm and stained with hematoxylin and eosin for light microscopic examination. Liver sections were examined for inflammation, loss or regeneration of tissue, and nuclear location. Muscle sections were examined for striation, nuclear changes, infiltration, hyaline and granular degeneration, and regeneration.

Statistical analysis. The data obtained from blood analyses was subjected to Mann-Whitney U test for two independent samples using the Minitab® Statistical Software. Growth parameters were examined by ANOVA. Levels of significance were determined using Tukeys HSD test, with critical limits being set at $P<0.05$.

Results

Growth performance, survival rate, and biochemical parameters of blood serum of fish fed with the two diets, as well as individual body weight, weight gain, survival rate, and FCR are given in Table 2. Mean individual body weight ranged from 359.32±58.36 to 431.17±68.72 g. Mean weight gain and FCR were 187.21±45.17g and 2.29±0.19 for the control group, and 259.06±55.53 g and 2.23±0.17 for the treated group, respectively. There were significant differences of diet between the groups on body weight and weight gain ($P<0.05$) but there was no significant effect on the survival rates and FCR ($P>0.05$) at the end of the experimental period.

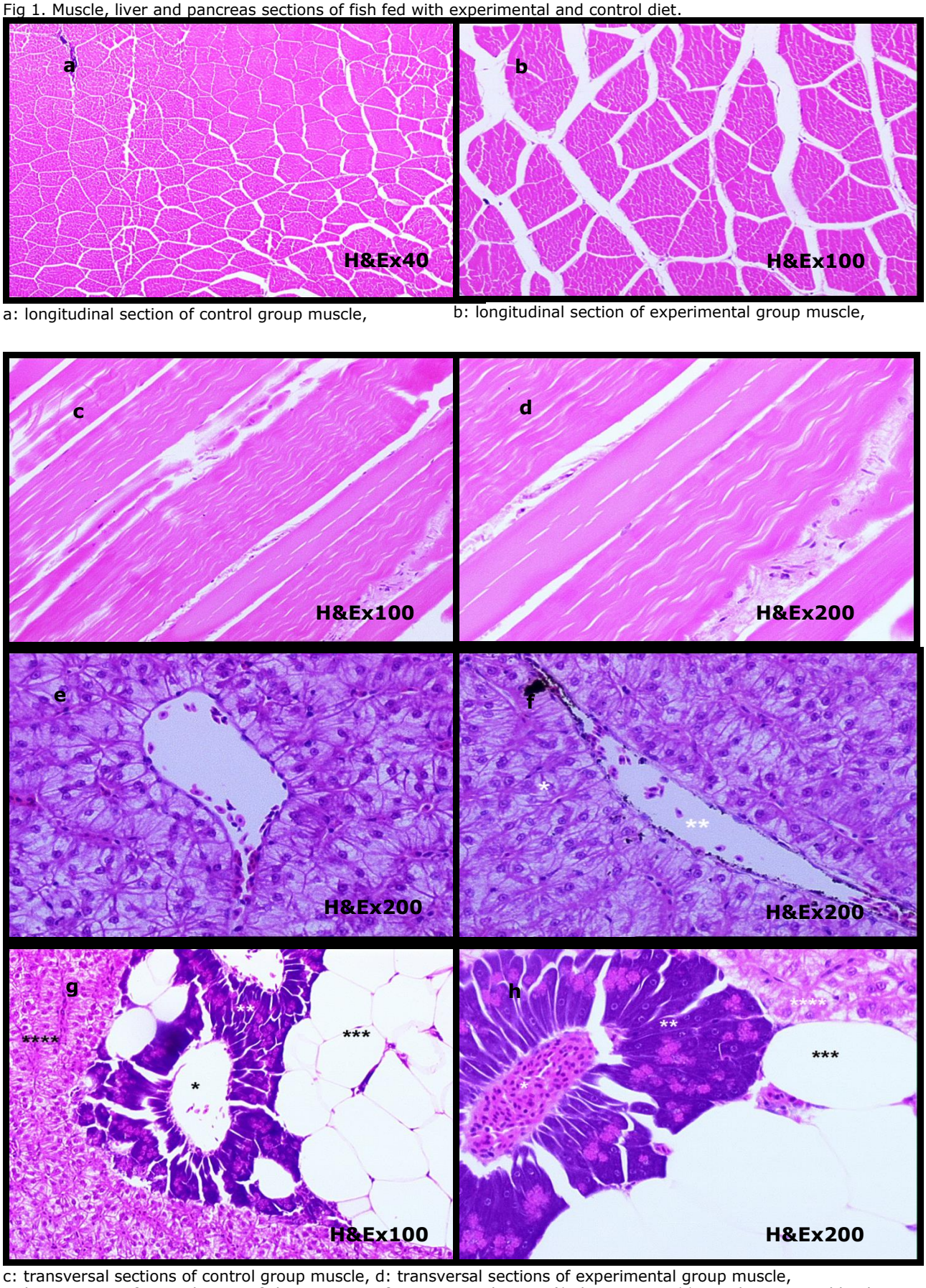
Table 2. Growth and biochemical parameters of fish

Variable	*Diets*	
	Control diet	*Experimental diet (2 g/kg MOS)*
Initial body weight (g)	172.11±13.19	172.11±13.19
Final body weight (g)	359.32±58.36[a]	431.17±68.72[b]
Weight gain (g)	187.21±45.17[a]	259.06±55.53[b]
Food conversion ratio (FCR)	2.29±0.19	2.23±0.17
Survival rate (%)	99.3	99.4
AST (IU/l)	98±5	102±7
ALT (IU/l)	20.05±2	18.03±1.9
ALP (IU/l^{-1})	121.75±16.75	163±13
PO_4 (mg/ dl)	9.8±0.7	9.4±0.9
Fe (µg/dl)	73±11.6	63±6.8
Na (mmol/l)	174.38±6.25	181.25±2.25
K (mmol/l)	6.36±0.71	6.41±0.44
Mg (mmol/l)	2.2±0.5	2.1±0.2
Ca (mg/dl)	13.51±0.63	13.93±0.18
Cl (mmol/l)	158±5.38	156±3.5
TP (g/dl)	3.29±0.29	3.21±0.24
GLC (mg/dl)	115.25±12.25	159±11.63
CHOL (mg/dl)	264±41	291±24
TRIG (mg/dl)	736±28	755±42
CREA (mg/dl)	0.10±0.02	0.11±0.01
DBIL (mg/dl)	0.09±0.03	0.08±0.01
IBIL (mg/dl)	0.30±0.10	0.35±0.05
URICA (mg/dl)	0.60±0.15	0.60±0.10
URE (mg/dl)	11.25±1.38[a]	17.50±1.13[b]

Abbrevations are as follows: AST= aspartate amino transferase, ALT= alanine amino transferase, ALP= alkaline phosphatase, PO_4= phosphate, Fe= iron, Na= sodium, K= potassium, Mg= magnesium, Ca= calcium, Cl= chloride, TP= concentration of total protein, GLC= glucose, CHOL= cholesterol, TRIG= triglyceride, CREA= creatinine, DBIL= direct bilirubin, IBIL= indirect bilirubin, URICA= uric acid and URE= urea.
The values are expressed mean ± SDs (n=1.000 for the growth parameters and n=10 for the blood analysis).
Significantly different from the control ($P< 0.05$).

The histology of muscle, liver, and pancreas, from fish fed the experimental diet did not differ from control, and displayed normal morphological characteristics (Figure 1).

Fig 1. Muscle, liver and pancreas sections of fish fed with experimental and control diet.



a: longitudinal section of control group muscle, b: longitudinal section of experimental group muscle,

c: transversal sections of control group muscle, d: transversal sections of experimental group muscle,
e: liver section of control group, f: liver section of experimental group (*: liver tissue, **: erythrocytes in blood vessel), g: pancreas section of control group (*:erythrocytes in blood vessel, **: pancreas tissue, ***: lipid tissue, ****: liver tissue), h: pancreas section of experimental group (*:erythrocytes in blood vessel, **: pancreas tissue, ***: lipid tissue, ****: liver tissue)

Discussion

The present study showed that the addition of prebiotic mannanoligosaccharides at 2g/kg in a pelleted diet significantly increased weight gain in gilthead seabream. This result was similar to other studies done with rainbow trout (*Oncorhynchus mykiss*) (Staykov et al. 2007), seabass (*Dicentrarchus labrax*) (Torrecillas et al. 2007), European catfish (*Silurus glanis*), red tilapia (*Oreochromis mossambicus* x *Oreochromis urolepis*) (Denev et al. 2009), common carp (*Cyprinus carpio*) (Atar and Ateş, 2009), and gilthead seabream (*Sparus aurata*) (Gültepe et al. 2011; 2012). In contrast, MOS additives to feed did not have a significant effect on growth some fish species, such as gulf sturgeon (*Acipencer oxyrinchus desotoi*) (Pryor et al. 2003), turbot (*Scopthalmus maximus*) (Mahious et al. 2006), hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) (Genç et al. 2007) and Atlantic salmon (*Salmo salar*) (Grisdale-Helland et al. 2008). There are many differences in the biochemical parameters of blood between different fish species. These parameters may be affected by factors such as water temperature, age, sex, maturation period, seasons, food, diseases, and chemical elements. Duration of diseases, toxicological conditions, and feedstuffs may also cause variations (Aydın et al. 2000; Salnur et al. 2009; Bulut et al. 2010; Satheeshkumar et al. 2011). In this study, biochemical indices of the blood did not differ significantly between the control and the experimental group ($P<0.05$); blood urea levels generally increased in relation to high protein diets (Preston et al. 1965; Eggum 1970); protein efficiency of feed improved growth with the addition of MOS without affecting the FCR. No histological differences or pathological effects were observed between the tested groups.

In conclusion, the results of this study show that the addition of prebiotic MOS to gilthead seabream improved protein efficiency and increased weight gain.

Acknowledgements

This study was partially supported by Kastamonu University Research Fund (Project Number KÜBAP-01/2013-29), Kılıç Seafish Aquaculture Import-Export Co.-Turkey, and Alltech-Turkey.

References

AOAC (Association Official Analytical Chemists), 2000. Official Methods of Analysis, 17th edn. Arlington, VA.

Atar H.H. and M. Ateş, 2009. The effects of commercial diet supplemented with mannanoligosaccharide (MOS) and vitamin B_{12} on the growth and body composition of the carp (*Cyprinus carpio* L. 1758). *JAVA*, 8:2251-2255.

Aydın S., Gültepe N. and H. Yıldız, 2000. Natural and experimental infections of *Campylobacter cryaerophila* in rainbow trout: Gross pathology, bacteriology, clinical pathology and chemotherapy. *Fish Path.*, 35(3):117-123.

Aydın S., Erdoğan O. and A. Yıldırım, 2000. Aras nehrinde yaşayan *Capoeta capoeta capoeta* (Güldenstaedt, 1772)'nın kan glikoz düzeyindeki aylık değişimler. *Turk. J. Vet. Anim. Sci.,* 24:523-528.

Bulut M., Gültepe N., Mendeş M., Güroy D. and M. Palaz, 2010. According to canonical correlation, the evaluation of blue fish (*Pomatomus saltatrix*) blood chemistry. *JAVA,* 9(4):666-670.

Coz-Rakovac R., Strunjak-Petrovic I., Hacmanjek M., Popovic T.N., Lipej Z. and B. Sostaric, 2005. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the North Adriatic Sea. *Vet. Res.Com.,* 29:677-687.

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

Eggum B.O., 1970. Blood urea measurement as a technique for assessing protein quality. *J. Nutr.*, 24:983-988.

Ganguly S., Paul I., Mukhopadhayay S.K., 2010. Application and Effectiveness of Immunostimulants, Probiotics, and Prebiotics in Aquaculture: A Review. *The Israeli Journal of Aquaculture - Bamidgeh* 62(3), 130-138

Genc M.A., Yilmaz E., Genc E. and M. Aktas, 2007. Effects of dietary mannanoligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). *Isr. J. Aquacult. - Bamidgeh,* 59:10-16.

Grisdale-Helland B., Helland S.J. and D.M. Gatlin III, 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galacto-oligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture*, 283:163-167.

Gouveia A. and S.J. Davies, 2000. Inclusion of an extruded dehulled pea seed meal in diets for juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture*, 182:183-193.

Gültepe N., Salnur S., Hoşsu B. and O. Hisar, 2011. Dietary supplementation with mannanoligosaccharides (MOS) from Bio-Mos enhances growth parameters and digestive capacity of gilthead sea bream (*Sparus aurata*). *Aquacult. Nutr.,* 17:482-487.

Gültepe N., Hisar O., Salnur S., Hoşsu B., Tanrıkul T.T. and S. Aydın, 2012. Preliminary assessment of dietary mannanoligosaccharides on growth performance and health status gilthead seabream *Sparus auratus*. *J. Aqua. Anim. Health*, 24:37-42.

Mahious A.S., Gatesoupe F.J., Hervi M., Metailler R. 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.

Newman K., 1994. Mannan-oligosaccharides: Natural polymers with significant impact on the gastrointestinal microflora and the immune system. Biotechnology in the Feed Industry, Proocedings of Alltech's Tenth Annual Symposium, (Ed. Lyons T.P. and K.A. Jacques), Nottingham University Press, Notthingham, UK, p. 167-175.

Preston R.L., Schnakenberg D.D. and W.H. Pfander, 1965. Protein utilization in ruminants: I. Blood urea nitrogen as affected by protein intake. *J. Nutr.* 281-288.

Pryor G.S., Royes J.B., Chapman F.A. and R.D. Miles, 2003. Mannanoligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in Gulf of Mexico sturgeon. *North Amer. J. Aquacult.,* 65:106-111.

Salnur S., Gültepe N. and B. Hoşsu, 2009. Replacement of fish meal by yeast (*Saccharomyces cerevisiae*): Effects on digestibility and blood parameters for gilthead sea bream (*Sparus aurata*). *JAVA,* 8(12):2557-2561.

Satheeshkumar P., Ananthan G., Kumar D.S. and L. Jagadeesan, 2011. Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. *Comp. Clin. Pathol.,* DOI 10.1007/s00580-011-1259-7.

Staykov Y., Spring P., Denev S. and J. Sweetman, 2007. Effect of a mannanoligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Int.,* 15:153-161.

Sweetman J. and S. Davies, 2006. Improving growth performance and health status of aquaculture stocks in Europe through the use of Bio Mos®. Nutritional Biotechnology in the Feed and Food Industries, Proocedings of Alltech's 22nd Annual Symposium, (Ed. Lyons T.P., Jacques K.A. and J.M. Hower), Nottingham University Press, Notthingham, UK, p. 445-452.

Torrecillas S., Makol A., Caballero M.J., Montero D., Robaina L., Real F., Sweetman J., Tort L. and M.S. Izquierdo, 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannanoligosaccharides. *Fish and Shellfish Immun.,* 23:969-981.

Yilmaz E., Genc M.A. and E. Genc, 2007. Effects of dietary mannanoligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. *Isr. J. Aquacult., - Bamidgeh,* 59:182-188.