

COMBINATIONAL EFFECTS OF MANNAN (MOS) AND CHITOSAN ON SURVIVAL, GROWTH PERFORMANCE, FEED UTILIZATION, BODY COMPOSITION AND INTESTINAL HISTOLOGY OF SEA BASS (*DICENTRARCHUS LABRAX*)

Zaki M¹. A., Salem² M. E., Gaber M. M³, Nour⁴ A. M.

^{1,4}Departments of Animal and Fish Production, Faculty of Agriculture, Alexandria University, El Shatby, Egypt.

²National Institute of Oceanography and Fisheries, Alexandria, Egypt.

³National Institute of Oceanography and Fisheries, Cairo, Egypt.

Corresponding Author: Nur Syairah Mohamad Aziz

ABSTRACT

This study was conducted in order to investigate the combined effect of dietary Mos and chitosan on sea bass fry (initial weight of 0.30±0.01 g/fish) were fed either control diet without Mos and chitosan or one of five dietary diets (0.5, 1.0, 2.0, 3.0 & 4.0 g/kg diet⁻¹). After 75 days feeding, the results showed that; the highest weight gain (WG) was recorded with fish fed diet 3 (1 g kg⁻¹ of Mos and Chitosan) and diet 4 (2 kg⁻¹ of Mos and Chitosan), compared with fish that were fed on the control diets. The best significantly survival rate was recorded at the fish were fed on the diet 3 compared with fish were fed diets 5 & 6. Feed conversion ratio (FCR) among the fish groups that were fed on Mos and Chitosan are significantly had FCR value better than the fish fed on the control diets. Protein efficiency ratio (PER) were be recorded significantly the highest value among the fish fed on the diets of 5 and 6 compared to the fish were fed on the control diet. Chemical composition of whole fish at the end of the experiment period showed that, the crude protein and gross energy was observed remarkably higher in the group of fish fed on diet 3. The results for dry mater were similar in all fish groups except for fish fed on diet 4 which had lower value. The crude fat value reaches maximum of fish fed on diet 1 and 2. In our study the intestinal microvillus length increased with increasing mixture of Mos and chitosan levels and became well developed at concentration levels 1 & 2 g kg diet⁻¹, while, at higher concentrations of mixture of Mos and chitosan, were highly developed to a degree of blocking the intestine pathway. In conclusion, Chitosan and Mos mixture diets could enhance the growth performance, Non-specific responses and reduce mortality in sea bass, higher than separated Chitosan and Mos diets due to synergistic effects of both compounds.

© Ideal True Scholar

KEYWORDS: Sea bass- Mos – Chitosan- Growth performance- intestine morphology

INTRODUCTION

European sea bass (*Dicentrarchus labrax*) is an important cultivated fish species with great economic interest. The most important farming countries are Greece, Turkey, Italy, Spain, Croatia, and Egypt (FAO, 2014). The annual production was more than 120,000 tonnes in 2010 (FAO, 2011). However, nutritional, environmental, or husbandry factors in intensification of aquaculture production systems expose fish to numerous stressors such as poor water quality, crowding, handling and transport, which may negatively affect their health (Houlihan *et al.*, 2001). Use of micro nutrients, for example vitamins, trace minerals, probiotics, and immunostimulants, as dietary supplements may benefit animal health, by improving performance, by improving the availability or utilization of nutrients through a variety of pathways (Staykov *et al.* 2007). Prebiotics, such as mannan oligosaccharides (Mos) have proved to be effective at enhancing health and growth

performance of fish (Staykov *et al.*, 2007; Torrecillas *et al.*, 2007; Dimitroglou *et al.*, 2010), improve gut morphology (Salze *et al.*, 2008; Dimitroglou *et al.*, 2010), improve disease resistance and stimulate the immune response of aquatic animals (Daniels *et al.*, 2010) and is widely used in nutrition as natural dietary supplementation to improve gastrointestinal health as well as overall health (Zhang *et al.*, 2012). Mos has also been demonstrated to benefit the gut health by improved absorption (Sang and Fotedar, 2010). One of the alternatives being considered today is the use of mannan oligosaccharides (Mos) as growth promoter as was recently highlighted by Ronel *et al.*, (2012).

Mannan oligosaccharide (Mos) is derived from cell wall of *Saccharomyces cerevisiae*, result from the partial hydrolysis of mannan polysaccharides (Quintero-Villegas, 2014). Ferket, (2004) described that when MOS is added to the animal diet, lectins of

these pathogens with mannose-specific Type-1-fimbriae are tricked into attaching to the mannan sugar instead of the carbohydrates attached to the intestinal villi. These lectins are then flushed out without being able to metabolize the sugar, resulting in a "cleansing" effect of the intestinal wall and preventing permanent damage to the villi (finger-like protrusions on the intestinal wall containing sites for nutrient absorption).

The positively effects of dietary mannan oligosaccharides (Mos) in European sea bass demonstrated in a lot of studies ; Torrecillas, (2013) indicated that dietary Mos (4 g kg⁻¹ Mos) enhances European sea bass (*Dicentrarchus labrax*) posterior gut epithelial defense by increasing membrane lipids content , promoting posterior gut health status and produce a higher feed intake. Also, It was found that, 0.2% and 0.4% of Mos in European sea bass (*D. labrax*) trial could growth was significantly improved at both inclusion levels The authors suggested that the increased growth may be related with enhanced amino acid absorption as demonstrated in chickens (Iji *et al.*, 2001). Sea bass (*D. labrax*) juveniles showed improved specific growth rate (SGR) and relative growth which increased significantly by about 10% after fed with MOS at 0.2 and 0.4% inclusion rates (Torrecillas *et al.*, 2006).

Chitosan is a natural cationic polysaccharide (Zhang *et al.*, 2010). It is a derivative of chitin and it is primarily produced from chitin by exhaustive alkaline deacetylation (Fouad, 2008). Chitosan has several favorable biological characteristics, as biodegradability, biocompatibility and non-toxicity (Kurita *et al.*, 1998). Additionally its ability to promote the growth performance, improve immune functions, inhibit intestinal tract microbial pathogens and lower cholesterol, etc. (Shi-bin and Hong 2012). So, nutritional significance of chitosan and the effectiveness of it as feed additives are considered (Hirano *et al.*, 1990).

Chitosan is an growth promoter active compound that is extracted from the shell of shrimp and crab, which is the essential component important for the growth of aquatic animals, so, when it was incorporated in the diet at a moderate level, it could promote the biosynthesis of the organism rapidly, which could highly enhancing the shrimp growth by frequency of moulting process, improving the digestion and absorption of nutrients at moderate levels, which result in high growth performance (Niu *et al.*, 2011). A number of studies have been conducted on effective utilization of chitosan as a fish feed supplement to promote growth, feed utilization and survival rate; The inclusion of chitosan at a level of 2% in the diet of common carp (*Cyprinus carpio*) enhanced the non-specific immunity, reduced the fish

mortality and enhanced the growth under stress conditions (Maqsood *et al.*, 2010). Geng *et al.*, (2011) investigated the effects of various levels of dietary *Bacillus subtilis* and chitosan on the growth performance, non-specific immunity and protection against *Vibrio harveyi* infection in cobia (*Rachycentron canadum*), he found that the combination of 1.0 g/kg⁻¹ *B. subtilis* and 6.0 g/kg⁻¹ chitosan is the best for the growth, innate immunity and disease resistance of cobia with an 8-week oral administration. The chitosan coated diet was also found to enhance the growth of the olive flounder (*Paralichthys olivaceus*) (Cha *et al.*, 2008).

Many studies showed that chitosan has immune stimulating action in different species of fishes (Siwicki *et al.*, 1994, Romeran *et al.*, 2002 and Cha *et al.*, 2008). Additionally, Meshkini *et al.*, (2012) found that, the adding chitosan at 0.25 % into the diet could stimulate the white blood cells activity and enhance resistance against some environmental stressors in rainbow trout (*Oncorhynchus mykiss*).

A combination of different carbohydrates showed synergistic effects on intestinal Ca absorption and balance in rats. The complexity and the variety of these later components may allow maintenance of high fermentation activity throughout the large intestine, which can increase their beneficial effects on mineral absorption (Younes *et al.*, 2001). In addition to, the combination of two different dietary fibers may result in a synergistic prebiotic effect, and may confer greater health benefits to the host throughout the entire colon (Rodríguez-Cabezas *et al.*, 2010). Prebiotic combination products and especially chewable Tablets as the preferred dosage form are described with high acceptability for the prophylaxis and treatment of inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), calcium and magnesium deficiency including osteoporosis, food intolerances, the regulation of the digestion, stimulation of the immune system, energy management, prophylaxis of colon cancer and protection of the gut microbial ecosystem in man. A prebiotic combination of inulin/oligofructose and resistant starch in very low amounts has been found to give a prebiotic effect. The low dosage of the two prebiotic components makes it possible to formulate inexpensive dosage forms with good compliance due to a small amount of ingredients. The small amount of the prebiotics also result in a virtually absence of side-effects.

To the author's knowledge no studies on Mos and Chitosan combination were carried out. So the objective of this study to evaluate combinational effects of mannan oligosaccharide (Mos) and chitosan on survival, growth performance, feed

utilization, body composition and intestinal histology of sea bass (*Dicentrarchus labrax*).

development followed the description of Holden and Raitt (1975).

MATERIAL AND METHODS

Experimental Diets

Formulation and proximate composition of the experimental diets are presented in Table (1). Mos and chitosan (obtained from National Institute of Oceanography and Fisheries Alexandria) was included in the diet at (0.5, 1.0, 2.0, 3.0 & 4.0 g kg⁻¹) and control without mixture of Mos and Chitosan based on the best results obtained before, in terms of growth performance, feed utilization and survival. The diets preparation and feeding system were as same as explained before in the first experiment.

Water Quality

Water quality parameters (temperature, dissolved oxygen, pH, ammonia, nitrate and nitrite) were monitored to ensure water quality remained well within limits recommended for Sea bass. Water temperature and dissolved oxygen were measured every other day using a YSI Model 58 oxygen meter (Yellow Springs Instruments, Yellow Springs, OH). Ammonia and nitrite were measured at weekly intervals using the titration methods of Golterman, *et al.*, (1978) pH was monitored twice weekly using an electronic pH meter (pH pen Fisher Scientific, Cincinnati, OH). During the feeding trial, the water quality parameter averaged (\pm SD): water temperature 27.8 \pm 0.8°C dissolved oxygen 6.8 \pm 0.4 mgL⁻¹; pH 7.4 \pm 0.6; ammonia 0.2 \pm 0.04 mgL⁻¹; nitrite 0.1 \pm 0.05 mgL⁻¹; nitrate 1.5 \pm 0.2 mgL⁻¹, salinity 35.2 \pm 1.1 gL⁻¹

Chemical Analysis of Diets And Fish Carcasses

Proximate analysis of the formulated diets and fish carcasses were determined according to standard methodology (AOAC, 2006). At the end of the experiment, six randomly sampled fish from each treatment were collected for carcass analysis. Crude protein content was determined by the Kjeldahl method using an Auto Kjeldahl System, crude lipid content by the Soxhlet extraction method, ash content by a furnace muffle (550 °C for 4 h), moisture content by a dry oven (105 °C for 24 h) and crude fiber content by an automatic analyzer (Fibertec, Sweden) (AOAC, 2006).

Histological Analysis

About 6 fish per treatment were sacrificed to obtain intestine. Intestine were fixed in Bouin's solution (Carson, 1992) for 24 hours and then transferred to alcohol (70%). After conventional histological processing, sections (5-7 μ m thick) were stained with haematoxylin and eosin and observed under a light microscope (Leica DM500). Assessment of intestine

Calculations and Statistical Analysis

Calculations of growth parameters were conducted according Cho and Kaushik (1985). Data were analyzed by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical analysis system 1993) Duncan's multiple range tests was used to compare differences among individual means. Treatment effects were considered significant at P<0.05. All percentages and ratio were transformed to arcsine values prior to analysis (Zar, 1984).

RESULTS

Growth Performance

The effect of mixture of Mos and Chitosan on Sea bass fry growth parameters is shown on Table (2). The results revealed that at the start of the experiment, there were no significant differences (P \leq 0.05) in average initial body weight, which indicates that there was homogeneity among these groups. At the end of the experiment, significantly (P \leq 0.05) the highest body final body weight (FBW), Weight gain and average daily gain (ADG) was recorded with fish fed diet 3 (1 g kg⁻¹ of Mos and Chitosan) and fish fed on diet 4 (2 g kg⁻¹ of Mos and Chitosan) followed by the fish that fed on diet 2 (0.5 g kg⁻¹ of Mos and Chitosan) and fish fed on diet 5 (3 g kg⁻¹ of Mos and Chitosan), compared with fish that were fed on the control diet, which had the lowest body final body weight (FBW), Weight gain and average daily gain (ADG). The groups of fish fed diet 2, 3, 4 & 5 had significantly (P \leq 0.05) the highest values of SGR and RGR compared with groups of fish fed control diet 1 and the fish that fed on the diet 6.

Survival Rate

The effect of mixture of Mos and Chitosan on the survival rate of sea bass is shown in Table (2). The best significantly survival rate was recorded at the fish were fed on the diet 3 followed by the fish that fed on diet control, 2 and 4 compared with fish were fed diets 5 and 6, which had the lowest survival rate.

Feed Utilization Efficiency

The effect of dietary treatments on sea bass fry were shown on Table (3). The fish that had fed on the diets 3 and 4 showed significantly the highest feed intake ratio (FI, g/fish) compared to fish fed on the control diets which had significantly the lowest FI value. There was no significant variation among feed conversion ratio (FCR) of the fish groups that were fed on diets 1(control), 3 and 4 but the all of them are significantly had FCR value varied from the fish fed on the diet 5 and 6. Protein efficiency ratio (PER) were be recorded significantly the highest value

among the fish fed on the diets of 5 and 6, compared to the fish fed on the diet 1, 2, 3 and 4 which significantly had the lowest PER value. Protein productive value (PPV) was the best at fish fed the diets 1 and 3, followed by the groups of fish fed on diets 2, 4, 5 and 6. The highest significant Energy gain (kcal) value was recorded at fish fed on diet 1, 2, 3 & 4 compared to other treatments. For Energy utilization Values, there was no significant variation among all treatments.

Fish Proximate Composition

Chemical composition of whole fish at the end of the duration of the experiment is shown in Table (4). A general tendency of increased crude protein was observed in fish fed on diet 1(control), followed by fish fed on diet 2&3, and fish fed on diet 6 had the lowest crude protein, the results for dry mater percentage were similar in all fish groups except for fish fed on diet 4 which had lower value, The crude Fat value reaches maximum was of fish fed on diet 1 and 2 followed by fish fed on diet 4. The lowest was recorded with fish fed on diet 3. For ash content, values were the highest for fish fed on diet 1, 2 and 3, followed by fish fed on diet 5, the lowest value was for fish fed on diets 4. Gross energy was the highest for fish fed on diet 3.

Histological Analysis

At the end of the experiment, the microscopic structure of sea bass intestines as affected by different levels of mixture of Mos and Chitosan supplement is shown in figures 1 (A-F). In our study the intestinal microvilli length increased with increasing Mos and Chitosan levels and reach maximum at concentration levels 1& 2 g kg diet⁻¹ as shown in Fig 1 (B&C) And the decreased small intestinal microvilli length of sea bass in this study when fed on control But it was observed, at higher Mos and Chitosan concentrations Figure 1, it was found that intestinal microvilli were highly developed to a degree of blocking the intestine which may prevent the mobility of the food through it.

DISCUSSION

The results of the present study indicated that Mos and Chitosan mixture supplementation in the diets of sea bass was helpful in improving growth rate, enhanced the nonspecific responses and reduced mortality, and the improvement mechanism may be partly attributed to improving morphological structure of small intestine. Histological analysis of intestine showed that the intestinal microvilli length increased with increasing Chitosan and Mos mixture levels, and became well developed at a concentration levels (1& 2 g kg diet⁻¹), which might lead to increase the absorptive surface area of intestine which means the increasing of nutrients absorption and as a result, enhanced the growth performance of the fish. In the

same time, the intestinal microvilli length was poor developed with control diet, but at higher Chitosan and Mos mixture concentrations (3 & 4 gkg diet⁻¹), it was observed that intestinal microvilli were highly developed to a degree of blocking the intestine pathway which might hinder the movement of the food through it, consequently, it depressed the nutrients absorption in blood stream which negatively affected the growth performance of the fish, this may explain why the growth of sea bass fish decreased as the Chitosan and Mos mixture concentration increased in the sea bass diet in this study. But, Chitosan and Mos mixture may showed synergistic effects on enhancing the digestion and absorption of nutrients at lower levels in comparison to the results of the experiment 1 and 2 where Chitosan and Mos when used separately produced better growth rate, the nonspecific responses and reduced mortality than the combined mixture of Mos and Chitosan. A number of studies have been conducted, Mos could improve growth of European sea bass (*D. labrax*) (Iji *et al.*, 2001; Torrecillas *et al.*, 2007), European catfish (*Silurus glanis*) (Bogut *et al.*, 2006), rainbow trout (Staykov *et al.*, 2005), tilapia fry (Samrongpan *et al.*, 2008), freshwater crayfish (*Cherax destructor*) (Sang *et al.*, 2011) and European lobster (*Homarus gammarus*) (Daniels *et al.*, 2010). Green tiger prawn (*Penaeus semisulcatus*) (Genc *et al.*, 2007), Pacific white shrimp (*Litopenaeus vannamei*) (Zhang *et al.*, 2012), tropic spiny lobster (*Panulirus ornatus*) (Sang and Fotedar, 2010) and rainbow trout (*O. mykiss*) (Staykov *et al.*, 2007). Additionally, it was found that dietary Mos (4 g kg⁻¹ Mos) enhances European sea bass (*Dicentrarchus labrax*) posterior gut epithelial defense by increasing membrane lipids content , promoting posterior gut health status and produce a higher feed intake (Torrecillas *et al.*, 2013). In addition to, the dietary Mos could improve the survival and decrease the mortality of marron (*Cherax tenuimanus*) (Sang *et al.*, 2009), European catfish (*Silurus glanis*) (Bogut *et al.*, 2006) and European lobster (*Homarus gammarus*) (Daniels *et al.*, 2010) and rainbow trout (*O. mykiss*) (Staykov *et al.*, 2007).

Intestinal microvilli provide a vast absorptive surface area, the increase in microvilli length and/or density can increase nutrient absorptive ability (Daniels *et al.*, 2010). In the present study, that dietary Mos could significantly increase intestinal microvilli length of sea bass (*D. labrax*), which suggested that dietary Mos could improve its nutrient absorptive ability. These results are similar to other studies of European lobster (*Homarus gammarus*) (Daniels *et al.*, 2010), gilthead sea bream (*Sparus aurata*) (Dimitroglou *et al.*, 2010), rainbow trout (*Oncorhynchus mykiss*) (Dimitroglou *et al.*, 2009) and cobia larvae (*Rachycentron canadum*) (Salze *et al.*, 2008). Those studies shown that the application

of Mos in diets has promoted the health of epithelium as well as the micro villi of the gut indicated by a more complicated architectural gut structure with longer villi, and hence a large surface area for nutrient absorption. A number of studies have been conducted on effective utilization of Chitosan as an animal feed supplement. (Kono *et al.*, 1987) studied the effect of chitin, Chitosan and cellulose as supplemental diet on the growth of cultured red sea bream, Japanese eel, and yellow tail. The growth rate of the three fishes fed with a 10% chitin supplement was the highest, Feed efficiency in the red sea bream and Japanese eel fed a 10% chitin supplemented diet was also the highest. a Chitosan coated diet was also found to enhance the growth of the olive flounder (*Paralichthys olivaceus*) (Cha *et al.*, 2008). The inclusion of Chitosan at a level of 2% in the diet of fish enhanced the non-specific immunity, reduced the fish mortality and enhanced the growth of common carp (*Cyprinus carpio*) under stress conditions (Maqsood *et al.*, 2010). (Geng *et al.*, 2011). investigated the effects of various levels of dietary *Bacillus subtilis* and Chitosan on the growth performance, non-specific immunity and protection against *Vibrio harveyi* infection in cobia (*Rachycentron canadum*). He found that the combination of 1.0 g kg⁻¹ *B. subtilis* and 6.0 g kg⁻¹ Chitosan is the best for the growth, innate immunity and disease resistance of cobia with an 8-week oral administration.

In addition to, the adding Chitosan at 0.25 percent into the diet could enhance the hematological parameters and resistance against some environmental stresses in rainbow trout (Meshkini *et al.*, 2012). Chitosan could stimulate the white blood cells function and rainbow trout immune system. Also, the fish received 0.25% Chitosan in their diet significantly had less mortality rate at hypoxic stress

than other groups and the control (Meshkini *et al.*, 2012). (Gopalakannan and Arul, 2006) reported that the addition of 1% Chitosan to the growth of carp (*Cyprinus carpio*) resulted in a significant increase. The incorporation of Chitosan at a level of 2% in the diet of fish enhanced the non-specific immunity, reduced carp (*Cyprinus carpio*) mortality and enhanced the growth of fish under stress conditions (Maqsood *et al.*, 2010). (Niu *et al.*, 2011) stated that moderate Chitosan was benefit to the growth and survival of (*Litopenaeus vannamei*) and the optimum supplement of dietary Chitosan level should be between 2.13 and 2.67 g/kg⁻¹ diet.

To the author's knowledge no studies on Mos and Chitosan combination were carried out to support these findings. But in general, a combination of different carbohydrates showed synergistic effects on intestinal Ca absorption and balance in rats. The complexity and the variety of these later components may allow maintenance of high fermentation activity throughout the large intestine, which can increase their beneficial effects on mineral absorption (Younes *et al.*, 2001). In addition to, the combination of two different dietary fibers may result in a synergistic prebiotic effect, and may confer greater health benefits to the host throughout the entire colon (Rodríguez-Cabezas *et al.*, 2010).

CONCLUSION

In conclusion, Chitosan and Mos mixture diets could enhance the growth performance, Non-specific responses and reduce mortality in sea bass, higher than separated Chitosan and Mos diets due to synergistic effects of both compounds.

Table (1): Composition of a diet similar to a high quality commercial sea bass diet and experimental diets supplemented with various percentage of mixture of Chitosan and Mos feed to fry

Ingredients (%)	Diets					
	1	2	3	4	5	6
Fish meal (C.P.70%)	50.0	50.0	50.0	50.0	50.0	50.0
Soybean meal (C.P.44%)	19.0	19.0	19.0	19.0	19.0	19.0
Yellow corn meal	7.0	7.0	7.0	7.0	7.0	7.0
Rice bran	11.7	11.7	11.7	11.7	11.7	11.7
Fish oil	3.0	3.0	3.0	3.0	3.0	3.0
Sunflower oil	3.0	3.0	3.0	3.0	3.0	3.0
Vit. & Min premix ¹	2.0	2.0	2.0	2.0	2.0	2.0
Calcium diphosphate	1.0	1.0	1.0	1.0	1.0	1.0
Molasses ²	2.0	2.0	2.0	2.0	2.0	2.0
Colin	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin C	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Antitoxins	0.1	0.1	0.1	0.1	0.1	0.1
Enzymes	0.1	0.1	0.1	0.1	0.1	0.1
Mix. Mos & Chi (g/kg)	0.0	0.5	1.0	2.0	3.0	4.0
Proximate analyses (%) ³						
Dry matter	91.9	96.2	94.9	93.7	94.8	94.5
Crude protein	45.1	45.1	45.1	45.1	45.0	45.1
Crude fat	11.2	10.3	10.1	10.1	10.3	10.7
Ash	10.2	10.6	10.4	10.7	10.5	10.0
Crude fiber	3.2	3.3	3.4	3.8	3.8	3.5

NFE ⁴	30.3	30.7	30.9	30.8	30.9	30.8
Gross energy Kcal	484.8	478.1	476.9	476.0	478.3	482.1
N:C ratio (mg cp :kcal)	93.0	94.3	94.6	94.7	94.2	93.6

¹Vitamin and mineral premixed according to Madan, et al. (2009).

²Molasses was used as a binder and attractant according to El-Saidy and Gaber (1998).

³Values represent the mean of three sample replicates.

⁴NFE = 100 - (% protein + % fat + % fiber + % ash).

Table (2): Effects of mixture of Chitosan and Mos 1.on growth performances of Sea bass fed control diet and the experimental diets 2-6

Diets	Average (g/fish)		Gain (g/fish)	ADG (g/fish/day)	SGR (%/day)	RGR (%)	Survival (%)
	Initial	Final					
1		3.05±0.05 ^c	2.84±0.1 ^c	0.037±0.001 ^c	3.55±0.1 ^b	1436.5±112.0 ^b	90.0±5.0 ^b
2		3.38±0.04 ^b	3.16±0.1 ^b	0.042±0.001 ^b	3.70±0.1 ^a	1610.2±72.0 ^a	93.0±2.9 ^b
3		3.68±0.04 ^a	3.46±0.02 ^a	0.046±0.002 ^a	3.80±0.1 ^a	1728.5±105.5 ^a	100.0±0.0 ^a
4		3.59±0.11 ^a	3.36±0.1 ^a	0.045±0.002 ^a	3.68±0.1 ^a	1587.9±122.0 ^a	93.33±2.9 ^b
5		3.4±0.05 ^b	3.19±0.3 ^b	0.043±0.001 ^b	3.71±0.1 ^a	1622.7± 68.8 ^a	86.67±2.9 ^c
6		3.01±0.05 ^c	2.80±0.2 ^c	0.037±0.001 ^c	3.55±0.1 ^b	1433.3± 52.8 ^b	85.0±0.0 ^c
F	0.84	60.16**	56.36**	56.28**	4.65*	4.66*	10.57**
M.S.	0.60156	0.00373	0.00389	0.0000007	0.00627	8546	8.33

¹Values are mean ± standard deviation. Values in the same column with same superscripts are not significantly different. IBW, initial body weight. FBW, final body weight. SGR (% per day), specific growth rate = (ln FBW/ln IBW)/time days /75).

Table (3): Effects of mixture of Chitosan and Mos. on feed utilization of Sea bass fed control diet and the experimental diets 2-6

Diets	Feed intake g/fish	FCR	PER	PPV%	Energy gain (kcal)	Energy utilization
1	6.08±0.017 ^c	2.14±0.10 ^b	1.04±0.05 ^b	19.42±1.15 ^a	4.65±0.05 ^a	15.81±0.64
2	6.65±0.12 ^b	2.11±0.04 ^b	1.06±0.01 ^b	16.50±1.47 ^b	4.69±0.46 ^a	14.76±1.35
3	7.25±0.29 ^a	2.10±0.07 ^b	1.06±0.04 ^b	17.9±0.8 ^a	4.98±0.07 ^a	14.42±0.9
4	7.06±0.09 ^a	2.10±0.05 ^b	1.06±0.02 ^b	16.98±0.07 ^b	4.88±0.19 ^a	14.52±0.29
5	6.36±0.09 ^b	1.98±0.01 ^a	1.12±0.01 ^a	17.82±0.60 ^b	4.53±0.29 ^{ab}	14.93±0.82
6	6.55±0.07 ^b	1.98±0.02 ^a	1.12±0.02 ^a	17.21±0.50 ^b	3.93± 0.19 ^b	14.72±0.71
F	51.37**	4.60*	4.91*	3.91*	6.44**	1.05
MS	0.0239	0.00297	0.000761	0.783	0.0648	NS

¹Values are mean ± standard deviation. * = (P≤0.05) **=(P≤0.01)

Values in the same column with same superscripts are not significantly different.

FCR, feed conversion ratio=dry feed fed/body weight gain. FER=feed efficiency ratio. PER=protein efficiency ratio.

Table (4): Initial and final whole body composition (wet weight basis) of Sea bass fed control diet and 2-6 diets supplemented with Mixture of Mos and Chitosan as feed attractant.

Diets	Dry matter	Crude protein	Crude fat	Ash	Gross energy (kcal)
1	32.31±0.44 ^a	16.20±0.69 ^b	5.91±0.27 ^a	7.10±0.35 ^a	143.85±12.53 ^b
2	28.80±2.51 ^a	16.46±0.20 ^b	6.09±0.7 ^a	7.19±0.87 ^a	139.87±2.84 ^{bc}
3	28.44±0.35 ^a	18.09±0.31 ^a	4.98±0.18 ^d	6.72±0.08 ^a	157.81± 0.78 ^a
4	27.94±0.48 ^b	15.70±0.25 ^c	5.54±0.24 ^b	5.44±0.03 ^d	140.85±0.85 ^b
5	28.77±0.50 ^a	15.56±0.54 ^c	5.28±0.41 ^c	6.24±0.6 ^b	137.67±6.93 ^c
6	28.72±1.21 ^a	14.98±0.54 ^d	5.47±0.41 ^c	5.66±0.13 ^c	135.94±6.93 ^c
F	5.24**	16.54**	4.14*	9.61**	4.28*
MS	1.42	0.210	0.119	0.169	43.7

Means in the same row bearing different superscripts differ significantly at 0.05 levels. Values are means ± SD (N = 3 for whole wet body composition).

The values of initial whole body composition were as follows: dry matter 20.56 ± 0.4, crude protein 10.12 ± 0.1, crude fat 2.2 ± 0.1, ash 3.9 ± 0.01, and gross energy 105.48 ± 1.51 kcal/100 g

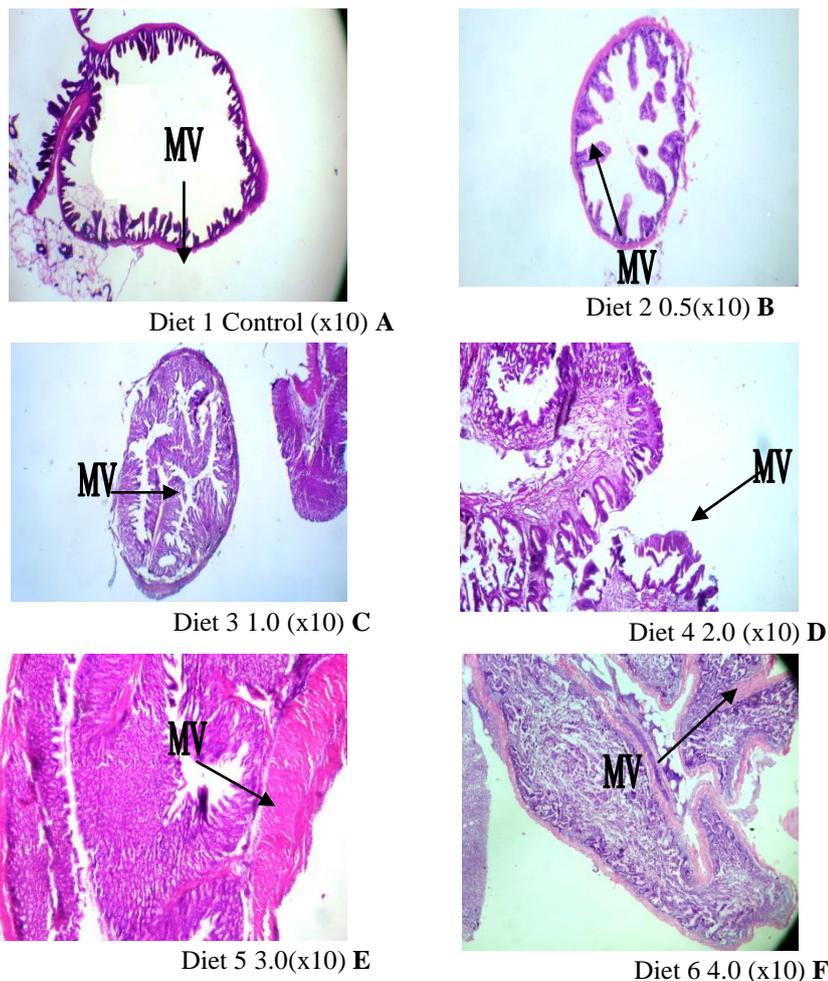


Fig. (1) Light photomicrograph of intestine sections (H&E) sea bass (*Dicentrarchus labrax*) fed on Mos and Chitosan.

REFERENCES

- AOAC. (Association of Official Analytical Chemists) (2006). Official Methods of Analysis. Horwitz W. 18th edition 2006, Washington, DC: 1018.
- Bogut I., Milakovic Z., Pavlicevic J. & Petrovic D (2006). Effect of Bio-Mos on performance and health of European catfish. In: Nutrition and biotechnology in the feed and food industries. Alltech's 22nd annual symposium (suppl. 1. abstracts of posters presented), Lexington, KY, USA.
- Carson F. (1992) Histotechnology: A Self-Instructional Text, pp 19, 1st ED, ASCP Press.
- Cho C. Y. & Kaushik, S. J (1985). Effect of protein intake on metabolizable and net energy values of fish diets. In: Nutrition and Feeding in Fish. (ed.) Cowey CB, Mackie AM, Bell JG) pp. 95-117. Academic Press, London, UK.
- Cha S.H., Lee J.S. and Song C.B., (2008). Effects of Chitosan-coated diet on improving water quality and innate immunity in the olive flounder, *Paralichthys olivaceus*. *Aquaculture*: 278 (110-118).
- Daniels C.L., Merrifield D.L., Boothroyd D.P., Davies S.J., Factor J.R. & Arnold K.E (2010). Effect of dietary *Bacillus* spp. and mannan oligosaccharides (Mos) on European lobster *Homarus gammarus* L. larvae growth performance, gut morphology and gut microbiology and gut microbiota. *Aquaculture*: 304 (49-57).
- Dimitroglou A., Merrifield L., Moate R., Davies J., Spring P. & Sweetman J (2009). Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of animal Science*: 87 (3226-34).

- Dimitroglou A., Merrifield L., Spring P., Sweetman J., Moate R. & Davies J (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).
- El-Saidy D.M.S. & Gaber M.M., (1998). Evaluation of the nutritional effects of using different levels of molasses in prepared practical diets for Nile tilapia *Oreochromis niloticus* L fry. *Menofiya Journal of Agriculture Research*: **23** (591-602).
- FAO (Food and Agriculture Organization of the United Nations) (2014). The State of World Fisheries and Aquaculture 2012. Rome. (1-40).
- FAO (Food and Agriculture Organization of the United Nations). (2011). The State of World Fisheries and Aquaculture 2010. Rome.
- Ferket P.R. (2004). Alternatives to antibiotics in poultry production: responses, practical experience and recommendations. In: Lyons TP, Jacques KA, editors. *Nutritional Biotechnology in the Feed and Food Industries*, Proceedings of Alltech's 20th Annual Symposium; 2004 July 17–20; Lexington, Kentucky. U. K.: Nottingham University Press (57–67).
- Fouad D. R. G. (2008). Chitosan as an antimicrobial compound: Modes of action and resistance mechanisms. Rheinischen Friedrich Wilhelm's Universität Bonn. PhD Thesis p. 2-18.
- Genc MA, Yilmaz E, Genc E. & Aktas M., (2007). Effect of dietary mannan oligosaccharid on growth, body composition and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus*×*O.aureus*). *The Israeli Journal of Aquaculture – Bamidgeh*: 59 (10–16).
- Geng X., Dong X., Tan B., Yang Q., Chi S., Liu H. & Liu X (2011). Effects of dietary Chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. *Fish & Shellfish Immunology*: 31(400-406).
- Gopalakannan A. & Arul V. (2006). Immunomodulatory effects of dietary intake of chitin, Chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture*: 255 (179-87).
- Golterman, H.L., Clymo, R.S. & Ohnstad M.A.M (1978) *Methods of physical and chemical analysis of fresh waters*. Blackwell Scientific Publications, Oxford, 214pp
- Hirano S., Itakura C., Seino H., Akiyama Y., Nonaka I., Kanbara N. and Kawakami T (1990). Chitosan as an Ingredient for Domestic Animal Feeds. *Journal of Agriculture and Food Chemistry*: 38 (1214-1217).
- Holden MJ, Raitt DFS (eds) (1975) *Manual de ciencia pesquera. parte 2. Métodos para investigar los recursos y su aplicación*. Doc Tec FAO Pesca 115, 1: 211 p.
- Houlihan D., Boujard T. & Jobling M (2001). *Food Intake in Fish*. Blackwell Science, Oxford
- Iji P.A., Saki A. A. & Tivey D.R (2001). Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. *Journal of Science and Food Agriculture*: 81 (1186–1192).
- Kono M., Matsui T. & Shimizu C (1987). Effect of Chitin, Chitosan and Cellulose as Diet Supplements on the Growth of Cultured Fish' in *Nippon Suisan Gakkaishi*: 53 (125-129).
- Kurita J., Nakajima K., Hirono I. & Aoki T (1998). Polymerase chain reaction (PCR) amplification of DNA of red sea bream iridovirus (RSIV). *Fish pathology*: 33 (17-23).
- Madan M., Bhanja S. K. & Yasmeeen B. (2009). Performance of chitin incorporated diet on the indigenous Kumaon Himalayan fishes: snow trout, *Schizothorax richardsonii* (Gray) and golden mahseer, *Tor putitora* (Hamilton) *Indian Journal of Fisheries*: 56:135-137.

- Maqsood S., Singh P., Samoon M. H. & Balange A. K, (2010). Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. International Aquatic Research 2: 77-85.
- Meshkini S., Taky A., Tukmechi A & Farhang-Pajuh F (2012). Effects of Chitosan on hematological parameters and stress resistance in rainbow trout (*Oncorhynchus mykiss*). Veterinary Research Forum, 3: 49 – 54.
- Niu J., Liu Y.J., Lin H.Z., Mai K.S., Yang H.J., Liang G.Y. & Tian L.X (2011). Effect of dietary Chitosan on growth and stress tolerance of postlarval *Litopenaeus vannamei*. Aquaculture Nutrition: 17: 406–412.
- Quintero-Villegas M., (2014). Non-digestible Oligosaccharides: Anti-adherence and Other Biological Properties. Dissertations & Theses in Food Science and Technology, University of Nebraska.
- Rodríguez-Cabezas M.E., Camuesco D., Arribas B., Garrido-Mesa N., Comalada M., Bailón E., Cueto-Sola M., Utrilla P., Guerra-Hernández E., Pérez-Roca C., Gálvez J. & Zarzuelo A., (2010). The combination of fructose oligosaccharides and resistant starch shows prebiotic additive effects in rats. Clinical Nutrition: 29: 832-9.
- Romeran K., Thu B. J. & Evensen O., (2002). Immersion delivery of plasmid DNA II. A study of the potential of a Chitosan based delivery system in rainbow trout (*Oncorhynchus mykiss*) fry. Journal of Control Release: 85(215-225).
- Ronel J., Conejos V., Acda S. P., Capitan S. S., Agbisit E. M. and Florinia E (2012). Merca2 Mannan Oligosaccharides from Yeast (*Saccharomyces cerevisiae*) Cell Wall Improves Nutrient Digestibility and Intestinal Morphology of Growing Pigs [*Sus domesticus* (Erleben)]. PHILIPP AGRIC SCIENTIST: 95 (305–311).
- Samrongpan, C., Areechon N., Yoonpundhan R. & Srisapome P. (2008). Effects of mannan oligosaccharide on growth survival and disease resistance of Nile Tilapia (*Oreochromis niloticus* L.) fry. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-18, 2008, Cairo, Egypt, pp: 345-353.
- Sang H. M., Ky T. L. & Fotedar R., (2009). Dietary supplementation of mannan oligosaccharide improves the immune responses and survival of marron, *Cherax tenuimanus* (Smith, 1912) when challenged with different stressors. Fish and Shellfish Immunology: 27(341-348).
- Sang H. M. & Fotedar R., (2010). Effects of mannan oligosaccharide dietary supplementation on performances of the tropical spiny lobster juvenile (*Panulirus ornatus*). Journal of Fish and Shellfish Immunology: 28(483–489).
- Sang H. M, Fotedar R. & Filer K. (2011). Effects of dietary mannan oligosaccharide on the survival, growth, immunity and digestive enzyme activity of freshwater crayfish, *Cherax destructor* Clark (1936). Aquaculture Nutrition: 17 (629-635).
- Salze G., Mclean E., Schwarz M. H., & Craig S. R. (2008). Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. Aquaculture: 274(148-152).
- Shi-bin Y. & Hong C (2012). Effects of dietary supplementation of Chitosan on growth performance and immune index in ducks. African Journal of Biotechnology: 11 (3490-3495).
- Siwicki A.K., Anderson D. P. & Rumsey G. L (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Veterinary Immunology and Immunopathology: 41(125-139).
- Statistical Analysis System. SAS/STAT user's guide release 6.03 edition. SAS Institute Inc. Cary, North Carolina, USA. 1993.
- Staykov Y., Denev S., & Spring P., (2005). Influence of dietary mannan oligosaccharides (Bio-Mos) on growth rate and immune function of common carp (*Cyprinus carpio* L.). Pages 431–432 in B. Howald and R. Flos, editors. Lessons from the past to optimize the future. vol. 35. European Aquaculture Society, Special Publication 35. EAS, Oostende, Belgium.
- Staykov Y., Spring P., Denev S. & Sweetman J (2007). effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Journal of Aquaculture International: 15(153–161).

Torrecillas S. & Izquierdo M. S., (2006). The effect of Bio-Mos on European sea bass (*Dicentrarchus labrax*) juveniles: growth performance and feed utilization. Proceedings of Alltechs 22nd Annual Symposium April 23-26, 2006. Lexington, KY, USA.

Torrecillas S., Makol A., Caballero M.J., Montero D., Robaina L., Real F., Sweetman J., Tort L. & Izquierdo M.S., (2007). Immune stimulation and improved infection resistance in european sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Journal of Fish and Shellfish Immunology: 23(969–981).

Torrecillas S., Makol A., Betancor M. B., Montero D., Caballero M. J., Sweetman J. & Izquierdo M. (2013). Enhanced intestinal epithelial barrier health status on Europe an sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish and Shellfish Immunology: 34 (1485-1495).

Younes H., Coudray C., Bellanger J., Demigne C., Rayssiguier Y. & Re´me´sý C (2001). Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats British Journal of Nutrition: 86(479–485).

Zar J.H., (1984). Biostatistician Analysis. Prentice Hall, Englewood Cliffs, New Jersey, USA

Zhang X. J., Xia W., Liu P., Cheng Q., Tahirou T., W. Gu & Li B., (2010). Chitosan Modification and Pharmaceutical/Biomedical Applications. Marine Drugs: 8 (1962-1987).

Zhang J., Liu Y., Tian L., Yang H., Liang G. and Xu D., (2012). Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology: 33(1027-1032).