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Effect of Dietary Prebiotic Mannan Oligosaccharide (MOS) on Growth Performance, Intestinal Microflora, Body Composition, Haematological and Blood Serum Biochemical Parameters of Rainbow Trout (*Oncorhynchus mykiss*) Juveniles

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ABSTRACT

The present study investigated the effects of prebiotic mannan oligosaccharide (MOS; activeMOS[®]) on the growth performance, intestinal microflora, body composition, haematological and serum biochemical parameters in rainbow trout juvenile (Oncorhynchus *mykiss*). After acclimation, fish $(1.67\pm0.006 \text{ g})$ were allocated into 12 tanks (25 fish per tank) and triplicate groups were fed a control diet or diets containing 1, 2.5 and 4.0 g MOS kg⁻¹. At the end of trial (60 days), blood samples were collected from caudal vein of 48 apparently healthy fish. Result showed that in level of 1 g MOS kg^{-1} acquired final weight, Body Weight Increase (BWI), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) were significantly higher than the other experimental groups (p < 0.05). The highest and the lowest growth performances were observed in 1 g MOS kg⁻¹ and control, respectively. The fish fed with 1 g MOS kg⁻¹ had highest but not significant (p>0.05) intestinal lactic acid bacterial count. By increasing the supplementation level of MOS, the content of crude protein increased (p<0.05). The result showed no significant differences in serum enzymes (ALT, AST, ALP) activity, glucose, cholesterol, triglyceride and total protein among treatments (p>0.05) but levels of lymphocyte in the group fed 1 g MOS kg⁻¹ was significantly higher than other groups (p<0.05). Also, a non significant elevation of White Blood Cell (WBC), haematocrit, hemoglobin and eosinophil levels was found in the fish fed diet 1.0 g MOS kg⁻¹ (p>0.05). The results indicated that dietary administration of mannan oligosaccharide at the level of 1 g kg⁻¹ can positively influence the growth performance, intestinal microflora, body composition and some blood parameters of juvenile rainbow trout.

Key words: Mannan oligosaccharide, growth, intestinal microflora, blood variables, rainbow trout (*Oncorhynchus mykiss*)

INTRODUCTION

Aquaculture industry considers the disease occurrence as the state of restriction to aquaculture production at which adversely affects economical development (Ibrahem et al., 2010). Hence, it is strongly recommended to improve feed formulation with natural feed ingredients in order to overcome such an issue. This is achievable by maximizing the nutrient retention, feed conversion ratio, nutrient digestibility, dietary nutrient balance and minimizing the fish mortality in antibiotic-free breeding conditions through the development of health-promoting diets. One other possible means of accomplishing this is to introduce supplements of prebiotics and probiotics into the diet (Reza et al., 2009). Accordingly, in recent years, veterinarians and nutritionists wisely prescribe the use of probiotics and prebiotics as a substitute for antibiotics (Gao et al., 2008). According to the literatures, Probiotics characterized as live microorganisms being in charge of general well-being of host after supervised consumption. The uses of probiotics in human and animal nutrition are well documented and recently have been applied to aquaculture. Subsequently, evidence of the beneficial effects of probiotics resulted in the general principle of prebiotics (Lin et al., 2012). Prebiotics have been defined as "Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of health-promoting bacteria in the intestinal tract" (Hoseinifar et al., 2010). In addition, prebiotics promote the growth of Lactic Acid Bacteria (LAB) that are beneficial to health and lessen the density of pathogenic micro-organisms (Reza et al., 2009). Among the established prebiotics, mannan oligosaccharide (MOS), is most commonly used as the dietary supplementation for fish and crustacean species (Sang and Fotedar, 2010). The use of functional feed additives such as MOS to improve growth and health performance in the aquaculture industry is increasingly important as who are demanded the eco-friendly production practices (Dimitroglou et al., 2009). MOS is a glucomannoprotein complex derived from the cell wall of yeast (Saccharomyces cerevisiae) (Sang and Fotedar, 2010). Mannan oligosaccharide (MOS) has been shown to improve the overall condition of alimentary canal by restricting the accumulation of agents of disease and strengthening the body immune system (Staykov et al., 2007). Several studies have demonstrated that prebiotic of mannan oligosaccharide (MOS) can improve the growth parameters, survival, hematological and biochemical parameters, gut morphology and modulate the intestinal microbiota in various aquatic species, including sea bass (Dicentrarchus labrax) (Torrecillas et al., 2007), rainbow trout (Oncorhynchus mykiss) (Staykov et al., 2007; Yilmaz et al., 2007; Dimitroglou et al., 2009), atlantic salmon (Salmo salar) (Grisdale-Helland et al., 2008); Nile tilapia (Oreochromis niloticus) (Samrongpan et al., 2008), rohu (Labeo rohita) (Andrews et al., 2009), sea bream (Sparus aurata) (Gultepe et al., 2011), japanese flounder (Paralichthys olivaceus) (Ye et al., 2011). Rainbow trout (Oncorhynchus mykiss) is one of the most commercially important species grown in Iran and all around the world. Hence, the objective of the experiments was to determine the effects of mannan oligosaccharide (MOS) as a prebiotic on growth performance, intestinal microflora, body composition, haematological and some biochemical parameters of cultured juvenile rainbow trout (Oncorhynchus mykiss).

MATERIALS AND METHODS

Experimental diets: Experimental diets were prepared by using the supplementation of 0 (control), 1.0, 2.5 and 4.0 g kg⁻¹ MOS (Active MOS[®]; Biorigin, Lencois Paulista, Sa¨o Paulo, Brazil) to a commercial trout diet (Faradane co., Iran) as it is shown in Table 1. Different levels of MOS were blended in a kitchen robot for homogenous mix and sufficient water (400 g kg⁻¹) was

Table 1: Proximate composition of the commercial diet

Ingredients	Dry weight (%)
Crude protein	46.01
Crude lipid	13.05
Ash	13.11
Moisture	11.27
NFE ^a	16.56
Gross energy (MJ kg ⁻¹) ^b	18.82

^aNFE: 100-crude protein (%)+crude lipid (%)+ash (%)+moisture (%), ^bGross energy (GE) (MJ/kg): crude protein (%)×23.6+crude lipid (%)×39.5+NFE (%)×17)

added to the mix to from soft dough. The obtained dough was passed through a mincer equipped with 1 mm diameter mesh size disk. The pellets were air dried at 40°C in an oven and stored at -4°C during the experiment.

Fish and feeding trial: Experiment was carried out in semi-intensive farm located in Mazandaran, Qaemshahr, Iran during 60 days. The fish were acclimated to 300 L tank $(1.2\times0.5\times0.5 \text{ m})$ and fed on a control diet for 7 days before the experiment began and then, 300 rainbow trout juveniles with an initial mean body weight of 1.67 ± 0.006 g were randomly distributed in to 12 tanks, with 25 fish in each tank, with three replicates per diet. Continuous aeration was provided to each tank through air stone connected to a central air compressor. Water quality parameters such as dissolved oxygen ($6.6\pm0.35 \text{ mg L}^{-1}$), temperature ($14.8\pm1.68^{\circ}$ C) and pH value (7.8 ± 0.18) were measured every day during the experimental period. During the trial, the fish were fed 6-8% body weight to apparent satiation 6 times per day (07:00, 09:30, 12:00, 14:30, 17:00 and 19:30 h). Uneaten food and feces were siphoned out before next feeding.

Growth performance and survival: Fish in each tank were weighed once every 2 weeks and counted to record the growth and determine the daily ration. Growth performance and feed utilization were calculated according to the following equation:

Body Weight Increase (BWI g) = final weight of fish (g)- initial weight of fish (g)

According to Tacon (1990):

Specific Growth Rate (SGR % day⁻¹) = $\frac{\ln \text{ final weight of fish-ln initial weight of fish}}{\text{Days of feeding}} \times 100$

According to Hevroy et al. (2005):

Feed Conversion Ratio (FCR) = $\frac{\text{Dry feed fed (g)}}{\text{Wet weight gain (g)}}$

According to Hevroy et al. (2005).

Intestinal bacteria analysis The analysis of autochthonous intestinal microbiota was conducted at the end of the nutrition trial. Three fish were sampled in each treatment and starved for 48 h prior to microbiological sampling. The fish were killed by physical destruction of the brain and the

skin washed in a solution of 0.1% benzalkonium chloride before opening the ventral surface with sterile scissor. Intestinal tract samples of fish were removed, weighed and suspended in sterile saline [0.85% (w/v) NaCl]. The suspension, serially diluted to 10^{-6} , thereafter, 0.1 mL of the solution was spread in triplicate on to Nutrient Agar (NA). DeMan, Rogosa and Sharpe (MRS) were also used to detect Lactic Acid Bacteria (LAB). All of the plates were incubated at room temperature (25°C) and examined for 5 days (Rengpipat *et al.*, 1998; Mahious and Ollevier, 2005) and finally, the number of colonies were counted. Identification of the samples was carried out according to Bergy's method (Peter and Sneath, 1986).

Chemical analysis of diets and fish carcasses: Proximate analyses of the diet and fish carcasses were determined according to standard methodology (AOAC., 1996). At the end of the experiment, 6 randomly sampled fish from each treatment were collected for carcass analysis. Crude protein content was determined by kjeldahl method using Auto Kjeldahl System, crude lipid content by Soxhlet extraction method, ash content by a furnace muffler (550°C for 4 h) and moisture content by a dry oven (105°C for 24 h).

Haematological and biochemical analyses: At the end of the feeding trial, about 2 mL blood was drawn from the caudal vein of four fish from each tank after they were starved for 24 h. Blood samples were promptly centrifuged at 2500 rpm for 5 min and the serum was removed with a disposable transfer pipette (Shakoori *et al.*, 1996). Blood serum was analyzed with an auto-analyzer for analysis of glucose, cholesterol, triglycerides, total protein, albumin and some liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities (Borges *et al.*, 2004). To study the hematological parameters, the blood samples were suspended in heparinized tube and then values of Red Blood Cell (RBC), White Blood Cell (WBC), haematocrit (Hct), haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil, lymphocyte, monocyte and eosinophil were measured (Feldman *et al.*, 2000).

Statistical analysis: By application of SPSS (version 18), the data were subjected to one-way analysis of variance (ANOVA) to determine the difference between the means and the significant differences were tested by Duncan's Multiple Range Test and the confidence level was set at 95%.

RESULTS

Growth performance and survival: The data relating to growth performance, feed utilization and survival of juvenile rainbow trout fed with the experimental diets for 60 day are shown in Table 2. Compared to the control treatment, 1.0 g MOS kg⁻¹ supplemented diets significantly (p<0.05) improved growth performance and feed utilization, including final weight, Body Weight Increase (BWI), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of fed rainbow trout.

Microbiota: Intestinal microbiota analyses are shown in Table 3. There were no significant differences in intestinal lactic acid bacteria among treatments (p>0.05). But the group tested with 1 g MOS kg⁻¹ had higher intestinal lactic acid bacterial count (p>0.05).

Proximate composition of fish: The results showed that even though, addition of supplementation level of MOS on to the diet significantly (p<0.05) increased, the mean values of crude protein, however, there was no significant difference among the MOS diets. Conversely, body

Table 2: Growth parameters and feed utilization of juvenile rainbow trout fed with diets containing different levels of mannan oligosaccharide

		1.0 (g)	2.5 (g)	4.0 (g)
Growth parameters	Control		(MOS kg ⁻¹)	
Initial weight (g)	0.018 ± 1.67	0.018 ± 1.67	0.018 ± 1.67	0.018 ± 1.67
Final weight (g)	$0.09\pm22.60^{\circ}$	0.23 ± 27.17^{a}	0.21 ± 25.86^{b}	0.15 ± 25.74^{b}
BWI (g)	$0.05\pm20.93^{\circ}$	0.26 ± 25.50^{a}	0.20 ± 24.20^{b}	0.15 ± 24.07^{b}
SGR (%/day)	$0.00\pm2.26^{\circ}$	0.00 ± 2.45^{a}	0.01 ± 2.4^{b}	0.00 ± 2.4^{b}
FCR	$0.00{\pm}1.1^{a}$	$0.05{\pm}0.96^{ m b}$	0.57 ± 1.03^{ab}	0.05 ± 1.03^{ab}

Data assigned with different superscripts indicate significant differences (p<0.05)

Table 3: Lactic acid bacteria (LAB) levels [log colony forming units (CFU) g^{-1} intestine] in the gut of juvenile rainbow trout fed with diets containing different levels of mannan oligosaccharide

		1.0 (g)	2.5 (g)	4.0 (g)
Log CFU/g	Control		(MOS kg ⁻¹)	
NA	0.22 ± 4.15	1.36 ± 4.13	0.58 ± 4.78	0.61 ± 5.03
MRS	2.25 ± 4.38	0.65 ± 4.79	0.36 ± 3.48	0.35 ± 3.92
D : 1	M			

Data are presented as Mean±SD

Table 4: Carcass proximate composition (% wet weight basis) of juvenile rainbow trout fed with diets containing different levels of mannan oligosaccharide

		1.0 (g)	2.5 (g)	4.0 (g)
Composition (%) body	Control	(MOS kg ⁻¹)		
Crude protein	0.08 ± 14.75^{b}	0.08 ± 15.22^{ab}	0.17 ± 15.57^{a}	0.31 ± 15.67^{a}
Crude lipid	0.35 ± 4.15^{a}	0.48 ± 3.16^{b}	0.17 ± 2.72^{b}	0.08 ± 2.85^{b}
Ash	0.09 ± 3.68^{a}	0.08 ± 3.77^{a}	0.06 ± 3.67^{a}	0.13 ± 3.70^{a}
Moisture	0.06 ± 75.14^{a}	0.09 ± 75.64^{a}	0.43 ± 75.64^{a}	$0.29{\pm}75.58^{\mathrm{a}}$

Data assigned with different superscripts indicate significant differences (p<0.05)

Table 5: Haematological and biochemical parameters of juvenile rainbow trout fed with diets containing different levels of mannan oligosaccharide

		1.0 (g)	2.5 (g)	4.0 (g)
Blood parameters	Control		(MOS kg ⁻¹)	
RBC (per/mm ⁶)	$0.59{\pm}0.11$	0.69 ± 0.13	0.59 ± 0.06	0.73±0.08
WBC (per/mm ³)	8.30 ± 1.31	14.60 ± 7.20	14.03 ± 4.50	15.66 ± 3.28
Haematocrit (%)	46.00 ± 7.21	52.00 ± 7.00	42.00 ± 3.60	47.33±6.65
Haemoglobin (g dL ⁻¹)	13.83 ± 2.36	15.73 ± 2.15	12.90 ± 1.15	14.26 ± 1.56
MCV (fl)	774.00 ± 26.96	754.33±87.79	705.66 ± 42.92	663.00 ± 49.38
MCH (pg)	232.33±16.04	228.00 ± 24.63	216.66 ± 7.63	229.66 ± 54.81
MCHC (%)	300.33 ± 14.43	302.33 ± 2.51	307.33 ± 8.14	302.33 ± 10.50
Neutrophil (%)	8.00 ± 1.00	5.33 ± 0.58	7.00 ± 1.73	6.00 ± 1.73
Lymphocyte (%)	90.00 ± 1.00^{ab}	91.66±2.51 ^a	89.33 ± 1.15^{b}	90.66 ± 1.15^{ab}
Monocyte (%)	$1.00{\pm}0.00^{\rm b}$	1.33 ± 0.57^{ab}	2.33 ± 0.57^{a}	$2.00{\pm}1.00^{ab}$
Eosinophil (%)	1.00 ± 0.00	1.67 ± 0.57	1.33 ± 0.57	1.33 ± 0.57
$ALT (u L^{-1})$	742.66±91.00	698.00 ± 64.81	821.00 ± 190.28	703.66±63.10
$AST (u L^{-1})$	47.66 ± 5.03	39.00 ± 5.56	53.66 ± 26.83	38.33 ± 5.77
$ALP (u L^{-1})$	506.00 ± 103.59	483.66 ± 58.62	418.00 ± 35.51	404.66 ± 40.15
Glucose (mg dL ⁻¹)	106.00 ± 16.82	103.33 ± 30.89	83.00 ± 24.75	79.33±6.11
Cholesterol (mg dL ⁻¹)	219.33 ± 41.42	243.33 ± 7.50	238.00 ± 2.64	193.66 ± 29.67
Triglyceride (mg dL ⁻¹)	228.66 ± 65.04	182.00 ± 10.39	203.33 ± 25.75	186.66 ± 15.37
Total protein (mg dL ⁻¹)	2.80 ± 0.39	$2.74{\pm}0.22$	3.30 ± 0.42	2.51 ± 0.48
Albumin (g dL^{-1})	$1.64{\pm}0.19^{ab}$	$1.67{\pm}0.14^{ m ab}$	$1.83{\pm}0.15^{a}$	1.40 ± 0.22^{b}

Data assigned with different superscripts indicate significant differences (p<0.05)

crude lipid content was higher in the fish fed the control diet (p<0.05). In terms of ash and moisture, no significant (p>0.05) differences were recorded among the treatments, though, fish fed with the 1.0 g MOS kg⁻¹ had highest ash content compared with other groups (p>0.05) (Table 4).

Blood parameters: The effect of dietary MOS supplementation on rainbow trout blood profiles is presented in Table 5. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) activities did not significantly (p>0.05) differ between treatments. Red

Blood Cell (RBC), Mean Corpuscular Volume (MCV), mean corpuscular haemoglobin content (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and neutrophile were not significantly (p>0.05) affected by dietary mannan oligosaccharide. However, lymphocytes level were significantly (p<0.05) higher in the 1.0 g MOS kg⁻¹ fed fish compared to the control fed fish. Also, a non significant (p>0.05) elevation of White Blood Cell (WBC), haematocrit, hemoglobin and eosinophil levels was found in the fish fed diet 1.0 g MOS kg⁻¹ compared to other treatments (p>0.05). In terms of glucose, cholesterol, triglyceride and total protein parameters, there was no significant (p>0.05) difference among the treatment groups but increasing the level of MOS from 2.5-4.0 g MOS kg⁻¹ in the albumin content was significantly (p<0.05) decreased.

DISCUSSION

Final weight and growth: Application of aquatic food supplements such as vitamins, minerals and prebiotics can turn out well to improve aquatic animal health and prevent diseases, in aquaculture system. Quoting from (Ibrahem et al., 2010), "The use of these functional additive have increased rapidly in the last 20 years, due, impart to limitations associated with the treatment of disease outbreaks in food fish and in another part, to increase disease resistance by causing up-regulation of host non-specific defense mechanisms against pathogenic microorganisms" (Ibrahem et al., 2010). Accordingly, MOS, which is extracted from yeast cell wall material is an alternative product to antibiotics. In the digestive tract, MOS prevent attachment and colonization of pathogenic bacteria and reduce adverse effects of microflora metabolites. Mannan oligosaccharide may also enhance health by stimulating antibody production (Cetin *et al.*, 2005). The results of the current study showed that the highest growth performance and feeding parameters including final weight, body weight increase, specific growth rate and feed conversion ratio were observed in $1.0 \,\mathrm{g}\,\mathrm{MOS}\,\mathrm{kg}^{-1}$ (p<0.05). The better growth performance in $1.0 \,\mathrm{g}\,\mathrm{MOS}\,\mathrm{kg}^{-1}$ supplemented diets may be due to the production of extracellular enzymes by the gut microflora (Andrews et al., 2009). Several studies have reported improved growth performance and feed utilization of fish fed dietary mannan oligosaccharide (Torrecillas et al., 2007; Staykov et al., 2007; Yilmaz et al., 2007; Grisdale-Helland et al., 2008; Samrongpan et al., 2008; Gultepe et al., 2011; Ye et al., 2011). Mannan oligosaccharide promotes the growth of beneficial lactic acid bacteria in the intestine and these bacteria help in inhibiting the growth of pathogens by producing bacteriocins (Andrews et al., 2009). Contrary to the present findings, there are some reports of negative effects of dietary mannan oligosaccharide on other fish species such as Gulf sturgeon (Acipenser oxyrinchus desotoi) (Pryor et al., 2003), African catfish (Clarias gariepinus) (Genc et al., 2006), Hybrid tilapia (Oreochromis niloticus×O. aureus) (Genc et al., 2007), Channel catfish, Ictalurus punctatus (Welker et al., 2007), Nile tilapia (Oreochromis niloticus) (Sado et al., 2008), sea bream (Sparus aurata) (Dimitroglou et al., 2010), Kutum (Rutilus frisii kutum) (Akrami et al., 2010) and Giant sturgeon (Huso huso) juvenile (Razeghi et al., 2012). These negative effects may be attributed to the inability of intestinal microbiota to ferment excessive levels of prebiotics and subsequent accumulation in the intestine which may be deleterious to the enterocytes (Soleimani et al., 2012).

Intestinal microflora: Digestive tract is considered as one of the major infection routes, hence, it is important to enhance the understanding of the importance of intestinal microbiota in fish (Ringo *et al.*, 2006). One potential mechanism by which prebiotics may have an effect on the fish's health is through the selective colonization of the GI domain by beneficial microorganism including *lactobacilli, bifidobacteria, Saccharomyces* sp. and some nonpathogenic strains of *E. coli*

(Buentello *et al.*, 2010). LAB have been considered beneficial residents of the fish's intestinal ecosystem by producing bacteriocins, which inhibit growth of certain fish pathogens and thus positively affect the host's microflora (Reza *et al.*, 2009). However, in the current study, there were no significant (p>0.05) differences in intestinal lactic acid bacteria among treatments. But the fish fed with 1 g MOS kg⁻¹ had higher intestinal lactic acid bacterial count (p>0.05). Spring *et al.* (2000) explained that MOS supplement can bind to certain gram–negative bacteria and prevent intestinal cloning, which is a bacteria removal mechanism from gut. Dimitroglou *et al.* (2009) by studying the structure intestine of juvenile rainbow trout fed MOS, found that dietary MOS improves intestine morphology and increased absorptive surface area by promoting mucosal folding. Akrami *et al.* (2010) evaluated the effects of different levels of dietary mannan oligosaccharides (1.5, 3.0 and 4.5 g kg⁻¹) on the kutum (*Rutilus frisii kutum*) fry and observed a non significant elevation on total viable counts in the intestinal tract of fish fed diet containing 1.5 g MOS kg⁻¹. Also Razeghi *et al.* (2012) found no significant difference in intestinal lactic acid bacteria between beluga (*Huso huso*) fed control and MOS supplementation (2.0 and 4.0 g kg⁻¹) diets.

Body composition: In addition to improving the health status of the animal, prebiotics may affect growth, feed utilization and body composition. It needs to be taken into account that the results are not decisive and may be influenced by the prebiotic chosen, species, dietary supplementation level and duration of use (Grisdale-Helland *et al.*, 2008). The protein concentration in the body may be affected by dietary prebiotics, although the response seems to differ depending on the animal species (Genc et al., 2007; Torrecillas et al., 2007; Yilmaz et al., 2007). The results of present study showed that by increasing the supplementation level of MOS, the mean values of crude protein increased (p<0.05). Similarly, in rainbow trout (Oncorhynchus mykiss) and hybrid tilapia (Oreochromis niloticus×O. aureus), the body protein concentration has been reported to increase as the level of MOS was increased in the diet from 1.5 to 4.5 g kg⁻¹ (Genc *et al.*, 2007; Yilmaz *et al.*, 2007). Conversely, there are some other studies conducted by Dimitroglou et al. (2010) and Gultepe et al. (2011) on gilthead sea bream (Sparus aurata) that were fed with 2 and 4 g kg⁻¹ mannan oligosaccharide, Akrami et al. (2010) on Kutum (Rutilus frisii kutum) fry stage that treated with different levels of dietary mannan oligosaccharide (1.5, 3 and 4.5 g kg⁻¹) and Razeghi *et al.* (2012) on giant sturgeon (Huso huso) juvenile that were fed with 2 and 4 g kg⁻¹ mannan oligosaccharide, all reported no significant differences in body composition between control fish and those fed MOS supplementation diets.

Haematology and biochemistry: Haematological and biochemical parameters are considered as useful measuring instruments in order to check the quality of fish health and physiological responses, nutrient absorption and animal surroundings that affecting fish (Hoseinifar *et al.*, 2011a). Measuring the activity of various enzymes in fish can be used for confirming maturity and monitoring any changes in the waters quality (Shahsavani *et al.*, 2010). In the present study, serum ALT, AST and ALP levels were not affected by dietary mannan oligosaccharide (p>0.05). This result was in agreement with Ahmdifar *et al.* (2011) and Hoseinifar *et al.* (2011b) who reported that prebiotic inulin and oligofructose had no effects on these enzymes in beluga (*Huso huso*) serum, respectively. The results of the present study showed that dietary mannan oligosaccharides had no effects on RBC, MCV, MCH and MCHC. However, haematocrit, hemoglobin and WBC levels, particularly lymphocyte and eosinophil, were elevated in fish fed 1 g MOS kg⁻¹ (Table 5). This was similar to the results of Andrews *et al.* (2009) who observed a significant improvement in WBC,

RBC and Hb, in rohu (Labeo rohita) fed MOS supplemented diet in comparison with those fed on the control diet. On the contrary, Hisano et al. (2007) using at least 2% dehydrated yeast (principal source of MOS) inclusion in Nile tilapia (Oreochromis niloticus) diet found no effects on haematology. In 2007, Welker and his colleagues reported that 0.2% dietary mannan oligosaccharides had no effect on the WBC, RBC, Hct, Hb and total serum protein of Channel Catfish (Ictalurus punctatus) (Welker et al., 2007). Sado et al. (2008) also reported that 0.2-1.0% MOS had no effect on tilapia (Oreochromis niloticus) RBC, WBC, Hb, Hct, MCV, MHC, MCHC or plasma total protein. Razeghi et al. (2012) who evaluated the effects of different levels of dietary mannan oligosaccharides (2.0 and 4.0 g kg⁻¹) on the beluga (Huso huso) juvenile observed that there were no significant differences in hematological parameters between control and MOS treatment groups. The present study also showed that there were no significant differences in serum biochemical parameters such as glucose, cholesterol, triglyceride and total protein between treatments except on albumin. Andrews et al. (2009) observed a significant improvement in, serum protein and albumin in rohu (Labeo rohita) fed MOS supplemented diet in comparison with those fed on the control diet. Sado et al. (2008) explained that in fish, the ectothermic animals, the influence of environmental and individual factors on hematological parameters can be noticeable. Sado et al. (2008) explained that in fish, the ectothermic animals, the influence of environmental and individual factors on hematological parameters can be noticeable. Ye et al. (2011) showed that dietary Fructo Oligosaccharides (FOS), mannan oligosaccharides (MOS) and Bacillus clausii decreased flounder cholesterol and triglyceride compared to the control. It appears that fluctuations in hematological and biochemical variables may be associated with characteristics of species, inclusion rates of MOS, ingredients of diets, rearing period, etc (Taati et al., 2011). The use of prebiotics as MOS to improve growth and health status in fish still needs further research for better explanation of contradictory results. The complexity of carbohydrate structure in yeast's cell wall, yeast's different strains, fermentation and processing methods processing methods can all alter their function. In addition, depending on MOS concentration, administration period and population status (age, sex, gonadal maturation) different results can be presented (Sado et al., 2008).

CONCLUSION

In conclusion, the present study indicates that dietary administration of mannan oligosaccharide (MOS) as a prebiotic at the level of 1.0 g kg^{-1} can positively influence on growth, intestinal microflora, body composition and some blood parameters of juvenile rainbow trout and this kind of prebiotic could be an appropriate complement in diet cultured rainbow trout.

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