

Effect of Dietary Mannan Oligosaccharide on Growth Performance, Survival, Body Composition and Some Hematological Parameters of Carp Juvenile (*Cyprinus carpio*)

¹Reza Akrami, ²Majid Razeghi Mansour, ¹Hossein Chitsaz and ³Rabeeh Ziaei

¹Department of Fisheries, Islamic Azad University, Azadshahr Branch, Azadshahr, Iran

²Iranian Academic Center for Education, Culture and Research (ACECR),
Mazandaran Branch, Sari, Iran

³Iranian Fisheries Organization, Golestan Province, Gorgan, Iran

Abstract: Effect of dietary Mannan Oligosaccharide (MOS; activeMOS[®]) on growth, survival, body composition and some hematological parameters in carp (*Cyprinus carpio*) Juvenile were investigated for 45 days. Basal diet were supplemented with 0 (Control), 1.0, 2.0 and 3.0 g kg⁻¹ MOS in a totally randomized design trial in triplicate groups. The experiment carried out in 100 L PVC tanks. About 20 juvenile carp with initially average weight 1.3±0.17 g were stocked in tanks and fed up a day. There were no significant differences in growth and feeding parameters between fish fed control and MOS supplementation diets (p>0.05). The highest and the lowest growth performance were observed in 1.0 g kg⁻¹ MOS and control treatment, respectively. There were no significant differences in survival rate and body composition among experimental groups (p>0.05). But, in group treated with 1.0 g kg⁻¹ MOS showed higher protein carcass (p>0.05) than other group. An elevation of hematocrit, lymphocyte (p<0.05), WBC, RBC, Hb and eosinophil (p>0.05) were found in the fish fed diet 1.0 g kg⁻¹ MOS. The result indicated that 1.0 g kg⁻¹ MOS can improved growth performance, survival, final production and some blood parameters of carp juvenile and it is appropriate for supplementation in the diet of cultured juvenile carp.

Key words: Mannan oligosaccharide, survival, body composition, hematological parameters, common carp (*Cyprinus carpio*)

INTRODUCTION

During the last 2 decades, traditional use of antibiotics in aquaculture has been criticized because of the potential development of antibiotic resistant bacteria, the presence of antibiotic residues in seafood, the destruction of microbial populations in the aquacultural environment and the suppression of the aquatic animals immune system (Ringo *et al.*, 2010). As an alternative strategy to antibiotics, prebiotics have recently attracted extensive attention in aquaculture. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). This leads to increased growth rate (improvement of nutritional metabolism) and better health of the host (Ahmadifar *et al.*, 2009). Among the established prebiotics such as fructooligosaccharide, trans galactooligosaccharide, inulin and Mannan Oligosaccharide (MOS), MOS is the most commonly used

as the dietary supplementation for fish and crustacean species (Sang *et al.*, 2011). MOS are gluco mannoprotein complexes derived from the cell wall of yeast (*Saccharomyces cerevisiae*) (Sang *et al.*, 2011). These materials contain mannose as the primary carbohydrate element (Taati *et al.*, 2011). And have also been demonstrated to benefit the gut health by improved absorption and immune modulation in the target species (Sang and Fotedar, 2010). Mannan oligosaccharide also provides mannose attachments and triggers the complement cascade, activating the immune system (Andrews *et al.*, 2009). The effects of MOS on the growth performance, hematological parameters and immune responses have been studied in several aquatic species, including; Gulf sturgeon, *Acipenser oxyrinchus* (Pryor *et al.*, 2003), African catfish, *Clarias gariepinus* (Genc *et al.*, 2006), Rainbow trout, *Oncorhynchus mykiss* (Staykov *et al.*, 2007; Yilmaz *et al.*, 2007; Dimitroglou *et al.*, 2009), European sea bass, *Dicentrarchus labrax* (Torrecillas *et al.*, 2007), Channel

catfish, *Ictalurus punctatus* (Welker *et al.*, 2007), Hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) (Genc *et al.*, 2007), Nile tilapia, *Oreochromis niloticus* (Sado *et al.*, 2008; Samrongpan *et al.*, 2008), Atlantic salmon, *Salmo salar* (Grisdale-Helland *et al.*, 2008), Rohu, *Labeo rohita* (Andrews *et al.*, 2009), *Sparus aurata* (Dimitroglou *et al.*, 2010; Gultepe *et al.*, 2011, 2012), Kutum, *Rutilus frisii kutum* (Akrami *et al.*, 2010), Japanese flounder, *Paralichthys olivaceus* (Ye *et al.*, 2011) and Beluga, *Huso huso* (Razeghi *et al.*, 2012). Despite numerous studies of MOS-type prebiotics in many fish species, limited information is available on the effects of mannan oligosaccharide on carp. Common carp (*Cyprinus carpio*) is an important commercially produced fish all over the world. In many countries rearing of carp species is conducted using extensive and semi-intensive production technologies. Thus, the aim of this research was to evaluation the effects of Mannan Oligosaccharide (MOS) as a prebiotic on growth parameters, survival, body composition and some hematological parameters of juvenile common carp (*Cyprinus carpio*).

MATERIALS AND METHODS

Experimental diets: To prepare the diets, a commercial carp diet (containing 35% protein and 12% lipid) was mixed with the supplementation of 1.0, 2.0 and 3.0 g MOS (ActiveMOS[®]; Biorigin, Lencois Paulista, Sao Paulo, Brazil) and water and made again into pellets which were allowed to dry and stored at 4°C. Control diets were prepared adding only water (Table 1).

According to Tacon (1990):

$$\text{Body Weight Increase (BWI, g)} = \text{Final weight of fish} - \text{Initial weight of fish}$$

Bekcan *et al.* (2006):

$$\text{Percent Body Weight Increase (PBWI, \%)} = \frac{(\text{Final weight of fish} - \text{Initial weight of fish}) \times 100}{\text{Initial weight of fish}}$$

Hevroy *et al.* (2005):

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

Xue *et al.* (2006):

$$\text{Daily Feed Intake (FI, \% BW/day)} = \frac{100 \times \text{Total dry feed intake per fish}}{(\text{Initial fish weight} - \text{Final fish weight})^{0.5} \times \text{No. of days fed}}$$

Hevroy *et al.* (2005):

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

Fish culture and feeding regime: Common carp (*Cyprinus carpio*) juveniles were obtained from kahak Propagation and Rearing Center (Qom Province, Iran) and stocked in the PVC tanks (100-l) for 1 week before the beginning of the experimental regime. They (with a mean body weight of 1.3±0.17 g) were randomly allocated to 12 tanks with 20 fish in each tank with 3 replicates per diet. Continuous aeration was provided to each tank through air stone connected to a central air compressor. Water temperature, dissolved oxygen and pH were monitored daily and maintained at 25.9±3.3°C, 5.3±0.6 mg L⁻¹ and 6.9±0.5, respectively. During the trial, the fish were fed 4-6% body weight to apparent satiation 3 times daily (08:00, 14:00 and 20:00 h) for 45 days. Uneaten food and feces were siphoned out before next feeding.

Growth performance calculations: Fish in each tank were weighed every 2 weeks and counted to record growth and determine the daily ration. Growth performance and feed utilization of fish were calculated according to following equations:

Table 1: Proximate composition in the commercial diet

Ingredients	Percentage
Crude protein	35.0
Crude lipid	12.0
Ash	8.0
Moisture	10.0
Crude fiber	5.0
NFE ^a	30.0
Gross energy (MJ kg ⁻¹) ^b	18.1

^aNFE = 100 - (Crude protein, % + Crude lipid, % + Ash, % + Fiber, % + Moisture, %); ^bGross Energy (GE) (MJ kg⁻¹) = (Crude protein, %×23.6 + Crude lipid, %×39.5 + NFE, %×17)

Bai (2001):

$$\text{Protein Efficiency Ratio (PER, g/g)} = \frac{\text{Wet weight gain (g)}}{\text{Protein fed (g)}}$$

Ai *et al.* (2006):

$$\text{Survival rate, \%} = \frac{100 \times (\text{Initial number of fish} - \text{Final number of fish})}{\text{Initial number of fish}}$$

De Silva and Anderson (1995):

$$\text{Biomass Gain} = [\text{Final weight of fish (g)} - \text{Initial weight of fish (g)}] \times (\text{Number of final fish})$$

Chemical analysis of diets and fish carcasses: Proximate analysis of the formulated diets and fish carcasses were determined according to standard methodology (AOAC, 1990). 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 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, 1990).

Measuring hematological parameters: At the end of the trial, blood samples were taken from 3 fish per tank (i.e., n = 9 per treatment) from the caudal vein of carp juveniles after they were starved for 24 h. In order 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), Hematocrit (Hct), Hemoglobin (Hb), eosinophil, monocyte, lymphocyte and neutrophil were measured.

Statistical methods: The data were subjected to one-way Analysis of Variance (ANOVA) and if significant (p<0.05) differences were found, Duncan's multiple range test was used to rank the groups using the SPSS (Version 18).

RESULTS AND DISCUSSION

Growth performance of juvenile carp fed different levels of dietary mannan oligosaccharide are shown in Table 2. At the end of the study, there were no significant differences (p>0.05) in growth and feeding parameters between fish fed control and MOS supplementation diets but generally improved growth performance, feed conversion ratio and survival without significant difference were observed in carp fed on diets supplemented with 1.0 g kg⁻¹ MOS.

The proximate compositions of the different levels of dietary mannan oligosaccharide are presented in Table 3. There were no significant differences of body composition of carp juveniles from the different dietary

groups (p>0.05). However, fish fed with the 1.0 g kg⁻¹ MOS had highest protein and ash and lowest lipid content compared with other groups.

Effects of different dietary prebiotic mannan oligosaccharide levels on the hematological parameters are shown in Table 4. Hematocrit and lymphocyte increased significantly in the group treated with 1.0 g kg⁻¹ MOS compared with other groups (p<0.05). Also, a non-significant elevation of RBC, WBC, Hb and eosinophil levels were found in the fish fed diet 1.0 g kg⁻¹ mannan oligosaccharide (p>0.05).

The use of prebiotics, nondigestible dietary ingredients that beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of health promoting bacteria in the intestinal tract, is a novel concept in aquaculture (Akrami *et al.*, 2008).

This study investigate the effect of MOS on the growth and survival of common carp. MOS have been reported to increase growth in some terrestrial vertebrate animals (Savage *et al.*, 1997) and Crustacean (Genc *et al.*, 2007; Hai and Fotedar, 2009). The results of the present study showed that the highest growth performance and feeding parameters were observed in 1.0 g kg⁻¹ MOS. The positive effect of MOS, extracted from yeast cell wall, could be related to its ability to promote the growth of lactic acid bacteria in the intestine (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; Gridale-Helland *et al.*, 2008; Samrongpan *et al.*, 2008; Gulpepe *et al.*, 2011). Additionally also Ye *et al.* (2011), the effects of different levels of dietary fructo oligosaccharides, mannan oligosaccharides and *Bacillus clausii* on the Japanese flounder (*Paralichthys olivaceus*) were evaluated and explained that the weight gain rate and feed conversion ratio in fish fed the prebiotic and probiotic supplemented diet had significantly improved than in fish fed the control diet. On the contrary, several studies have revealed that growth parameters and feeding and survival have remained

Table 2: Growth performance of juvenile common carp fed diets containing varying levels of MOS (g kg⁻¹) after 45 day

Parameters	Control	1.0 g kg ⁻¹ MOS	2.0 g kg ⁻¹ MOS	3.0 g kg ⁻¹ MOS
Initial weight (g)	1.28±0.015	1.29±0.015	1.30±0.020	1.29±0.015
Final weight (g)	3.2±0.2000	3.64±0.350	3.50±0.150	3.60±0.300
BWI (g)	2.00±0.210	2.31±0.350	2.27±0.140	2.27±0.310
PBWI (%)	148.80±0.184	178.90±0.277	173.00±0.100	176.20±0.262
SGR (%/day)	2.10±0.170	2.43±0.230	2.39±0.870	2.41±0.210
Feed intake (%/day)	2.35±0.050	2.68±0.110	2.58±0.130	2.56±0.180
FCR (g)	3.75±0.610	3.16±0.200	3.73±0.450	3.23±0.570
PER (g/g)	1.77±0.120	1.94±0.160	1.78±0.110	1.85±0.210
Survival (%)	63.30±0.280	70.00±0.500	66.60±0.028	68.30±0.280
Biomass (g)	44.73±0.170	48.84±0.310	44.96±0.280	48.01±0.380

Data are presented as mean±SD

Table 3: Carcass composition (% wet weight basis) of juvenile common carp fed diets containing varying levels of MOS (g kg⁻¹) after 45 day

Body composition	Control	1.0 g kg ⁻¹ MOS	2.0 g kg ⁻¹ MOS	3.0 g kg ⁻¹ MOS
Crude protein	19.97±0.18	20.56±0.19	20.44±0.34	20.26±0.13
Crude lipid	1.64±0.30	1.61±0.30	1.67±0.20	1.68±0.10
Ash	2.95±0.21	2.96±0.20	2.91±0.23	2.90±0.25

Data are presented as mean±SD

Table 4: Hematological indices of juvenile common carp fed diets containing varying levels of MOS (g kg⁻¹) after 45 day

Parameters	Control (n = 9)	1.0 g kg ⁻¹ MOS (n = 9)	2.0 g kg ⁻¹ MOS (n = 9)	3.0 g kg ⁻¹ MOS (n = 9)
RBC (×10 ⁶ /mm ³)	1.20±0.11 ^a	1.29±0.15 ^{ab}	1.19±0.06 ^a	1.10±0.18 ^a
WBC (×10 ⁶ /mm ³)	12.90±1.31 ^{ab}	14.69±3.43 ^a	13.85±4.50 ^{ab}	13.25±3.28 ^a
Hct (%)	36.00±3.21 ^a	39.00±2.00 ^b	32.00±2.60 ^a	37.33±3.05 ^a
Hb (g dL ⁻¹)	8.23±2.36 ^{ab}	9.25±2.15 ^a	8.40±1.15 ^{ab}	8.26±1.56 ^{ab}
Eosinophil (%)	4.00±0.01	4.67±0.57	4.33±0.57	4.33±0.57
Monocyte (%)	2.33±1.52	3.07±0.01	3.33±2.30	3.00±1.73
Lymphocyte (%)	78.33±0.18 ^b	80.66±0.51 ^a	78.33±0.15 ^b	78.66±0.52 ^b
Neutrophil (%)	15.40±1.01	14.90±1.03	15.10±1.73	14.10±1.73

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

unaffected with mannan oligosaccharide applications in fish (Pryor *et al.*, 2003; Genc *et al.*, 2006, 2007; Welker *et al.*, 2007; Sado *et al.*, 2008; Dimitroglou *et al.*, 2010; Akrami *et al.*, 2010; Gultepe *et al.*, 2011; Razeghi *et al.*, 2012). The reasons for the different results are not clear yet. It may be because of the different basal diet, inclusion level, type of fructan, adaptation period, chemical structure (degree of polymerization, linear or branched, type of linkages between monometric sugars), origin of MOS, animal characteristics (Species, age, stage of production) and hygienic conditions of the experiment (Reza *et al.*, 2009). In the present study, survival rate increased in fish fed 1.0 g kg⁻¹ MOS (p>0.05). Hoseinifar *et al.* (2011) reported that it is possibly a sign of improved general health or immune status. However, further research is needed to determine their specific effects in the nutrition of fish. In addition to improving the health status of the animal, prebiotics may affect growth, feed utilization and body composition. The protein concentration in the body may be affected by dietary prebiotics, although the response seems to differ depending on the animal species (Grisdale-Helland *et al.*, 2008). The results revealed that fish fed with the 1.0 g kg⁻¹ MOS had higher protein content than the other group (p>0.05). Studies 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 showed different levels of dietary mannan oligosaccharide (0, 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 have no significant differences in body composition between fish fed control and MOS supplementation diets. But, 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 1.5-4.5 g kg⁻¹ (Genc *et al.*, 2007; Yilmaz *et al.*, 2007). There has been a lack of adequate research investigating the effects of dietary pre and probiotics administration on lipid metabolism in fish (Ye *et al.*, 2011). The result of present study showed that the fish fed with the 1.0 g kg⁻¹ MOS had lower lipid content than the other group (p>0.05). These phenotypes may be accredited to lipid metabolism alterations in the liver by short-chain fatty acids that improve the gastrointestinal tract environment (Ye *et al.*, 2011). To improve disease resistance and reduce the use of antibiotics, feed additives with immunostimulant

properties for example activation of White Blood Cells (WBC) and increasing of gut health have been extensively used in the husbandry of poultry and farm animals (Sado *et al.*, 2008). In the current study, hematocrit and lymphocyte increased significantly in the group treated with 1 g kg⁻¹ MOS compared with other groups (p<0.05). RBC, WBC, Hb and eosinophil were also higher in the fish feeding with 1 g kg⁻¹ MOS. Similar to these results, Andrews *et al.* (2009) observed a significant improvement in WBC, RBC and Hb in rohu (*Labeo rohita*) fed on the MOS supplemented diet in comparison with those fed on the control diet. On the contrary, Welker *et al.* (2007) reported that inclusion of MOS had no effect on hematological parameters (RBC, WBC, Hct and Hb) of Channel catfish (*Ictalurus punctatus*). Also Sado *et al.* (2008), Razeghi *et al.* (2012) and Gultepe *et al.* (2012) explained that dietary MOS had no significant effect on hematological parameters of Nile tilapia (*Oreochromis niloticus*), giant sturgeon (*Huso huso*) and Gilthead Seabream (*Sparus auratus*), respectively. It appears that fluctuations in hematological and biochemical variables may be associated to characteristics of species, inclusion rates of MOS, ingredients of diets, rearing period, etc (Taati *et al.*, 2011).

CONCLUSION

In this study, the result indicated that 1.0 g kg⁻¹ MOS can improved growth performance, survival, final production and some blood parameters of carp juvenile and it is appropriate for supplementation in the diet of cultured juvenile carp. This study encourages further research on different aspects of prebiotic administration in carp as well as immunological studies to determine the effects of prebiotics on the immune system and disease resistance of carp juveniles.

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