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Original Article

Dietary synbiotic improves the growth performance, survival and innate immune response of Gibel carp (*Carassius auratus gibelio*) juveniles

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Abstract: This study was conducted to evaluate the effect of dietary synbiotic (*Biomon imbo*) on the growth performance, survival and innate immune response of Gibel carp (*Carassius auratus gibelio*) juveniles. Fish with initially average weight 15.5±0.2 g were randomly distributed into tanks (20 fish/tank). Basal diets (Biomar) were supplemented with 0 (control), 0.5, 1.0, 1.5 and 2.0 g symbiotic per kg^{-1} in a totally randomized design trial in triplicate group. At the end of the experiment (60 days), innate immune parameters (serum Ig levels and lysozyme activity) and growth factors (final weight, weight gain (WG %), specific growth rate (SGR) and food conversion ratio (FCR)) were assessed. According to our results, the growth performance and feed efficiency improve in fish fed on the diet containing 2.0 g kg^{-1} synbiotic (P<0.05). There were no significant differences in survival among all treatments (P>0.05). Lysozym activity and serum Ig levels in fish fed the 2.0 g kg^{-1} synbiotic diet was higher compared to other experimental groups (P<0.05). These results indicate that synbiotic can be considered as a beneficial dietary supplement for improving the immune response and growth performance Gibel carp (*Carassius auratus gibelio*) juveniles.

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Introduction

Improvement and protection of fish health in commercial production practices is a major factor in the aquaculture industry. A novel approach to these goals is application of probiotics and prebiotics in fish farming industry (Irianto and Austin, 2002; Wang and Xu, 2006; Wang et al., 2008). Probiotic defined as organisms and substances which contribute to intestinal microbial balance and could enhance the growth and health of the host (Gatesoupe, 1999; Kesarcodi-Watson et al., 2008). Prebiotics are defined as non-digestible dietary ingredients that beneficially affect the host by selectively stimulating the growth and/or activating the metabolism of health-promoting bacteria in the gastrointestinal tract (Manning and Gibson, 2004). Gibson and Roberfroid (1995) have defined the mixture of pre- and probiotics as synbiotics that exert synergistic effects in promoting beneficial bacteria and the health of the gastrointestinal tract of the host, so their potential applications have spurred attention. Although benefits associated with prebiotics and probiotics are desirable, researchers are concerned about a conclusive result, depending on type and amount of pre- and probiotic consumed. Therefore more studies need to be conducted to provide a better understanding of their direct effects on health. The use of probiotics and prebiotics in aquaculture is now widely accepted but limited data is available regarding the application of synbiotics in aquaculture (Li et al., 2009; Rodriguez-Estrada et al., 2009; Daniels et al. 2010; Zhang et al., 2010; Ai et

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al., 2011; Ye et al., 2011; Mehrabi et al., 2012; Nekoubin et al., 2012; Nekoubin and Sudagar, 2012; Montajami et al., 2012). *Carassius auratus gibelio* is an improved strain of carp and suitable fish model for carrying out in vivo aquaculture experiments. Also, Gibel carp known as goldfish and one of the most popular ornamental species in the world due to its varieties with attractive body shape and skin color (Moreira et al., 2011). The aim of the present research was to study the effects of synbiotic (Biomin IMBO) on growth performance, Survival, and immune response of Gibel carp juveniles (*Carassius auratus gibelio*).

Materials and methods

Gibel carp juveniles (*Carassius auratus gibelio*) (average initial weight 15.5±0.2 g), obtained from a local ornamental fish farm (Golestan Province, Iran), were randomly stocked into 12 tanks (70 L) at a density of 20 fish/tank (3 tanks/treatment). Water temperature, dissolved oxygen and pH were monitored daily and maintained at 25.3±0.8°C, 5.2±0.3 mg/ L, and 7.24±0.15, respectively. Continuous aeration was provided to each tank through an air stone connected to a central air compressor.

The type of synbiotic was applied in this study was Biomin IMBO (Biomin, Herzogenburg, Austria) in which was comprised of probiotic (*Entercoccus faecium* 5×10^{11} CFU/g) and Fructooligosaccharide (FOS) as prebiotic. To prepare the diets, a commercial pellet diet (BioMar) (containing 41% protein, 6% lipid, 11.1% ash and 23.2 MJ/Kg GE) was mixed with the supplementation with various levels of synbiotic (0.5, 1.0 ,1.5 and 2.0 g/kg) and water, and made again into pellets (Cerezuela et al., 2008). The pelleted diets were air-dried, ground and sieved to produce a suitable crumble. Then the feed stored at 4°C until feeding trial. The experimental fish were weighted every 15 days in order to adjust the daily feed rate which was 3% of the total biomass the fish were fed three times daily to apparent satiation for 60 days.

In order to measure the growth parameters, weight and length of all fish were measured at every 15 days interval. After 8 week feeding period, Weight Gain (WG%), Specific Growth Rate (SGR %/day), Feed Conversion Ratio (FCR) and Survival Rate (%) were calculated according to following equations (Bekcan et al., 2006): $WG(\%) = (Wt-W_0) \times 100/W_0$, $SGR = (\text{Ln } Wt - \text{Ln } W_0) \times 100/t$, FCR = dry feed fed / Wet weight gain, , Survival rate = $(Nt/N_0) \times 100$. Here Wt and W_0 are final and initial body weights (g), respectively, t is duration of experimental days, N_0 is the initial number of fish and Nt is the final number of fish.

At the end of trial, six fish/tank were sampled for blood. In order to provide sufficient blood for the subsequent assays fish were euthanized, the tail cut off and blood was removed. For serum isolation, blood samples were aliquoted into non-heparinized tubes and left to clot for 12 h (at 4°C), prior to centrifugation at 5000 g (3500 rpm) for 5 min in a clinical centrifuge. Isolated serum was stored at -20°C until further analysis. Serum total immunoglobulin (Ig) levels were determined according to the method described by Siwicki and Anderson (1993). Briefly, serum total protein content was measured using a microprotein determination method (C-690; Sigma), prior and after precipitating down the immunoglobulin molecules, using a 12% solution of polyethylene glycol (Sigma). The difference in protein content represents the Ig content. Serum lysozyme activity was determined by turbidometric assay according to the method of Ellis with slight modifications (Ellis, 1990). Aliquots (1.75 m/L) of Micrococcus lysodeikticus suspension (Sigma) (0.375 mg/mL, 0.05 M PBS, pH 6.2) were mixed with 250 µ/L of each sample and the optical density was measured after 15 and 180 s by spectrophotometer (Biophotometer Eppendorf) at 670 nm. PBS was used as the blank and results were expressed in amounts of lysozyme µg/mg of sample calibrated using a standard curve determined with hen's egg white lysozyme (Sigma) in sterile sodium phosphate buffer.

Table 1. Growth and feed utilization of Gibel carp fed different dietary levels of synbiotic for 60 days

	Control	0.5 g kg^{-1}	1.0 g kg^{-1}	1.5 g kg^{-1}	2.0 g kg^{-1}
Final weight (g)	31.5 ± 2.5	34.8 ± 1.7	34.4 ± 2.9	35.5 ± 2.6	35.9 ± 3.12
WG (%)	54.2± 10.9	70.03 ± 10.2	69.06 ± 16.9	72.5 ± 13.6	73.16 ± 14.4
SGR (%/day)	0.48 ± 0.07	0.58 ± 0.06	0.56 ± 0.1	0.6 ± 0.08	0.62 ± 0.1
FCR	4.23 ± 0.8	3.3± 0.16	3.3 ± 0.75	3.2 ± 0.51	3.1 ± 0.84
Survival	90.2 ± 9.8	93.3± 6.6	96.6 ± 3.5	96.6 ± 3.5	97.6 ± 2.5

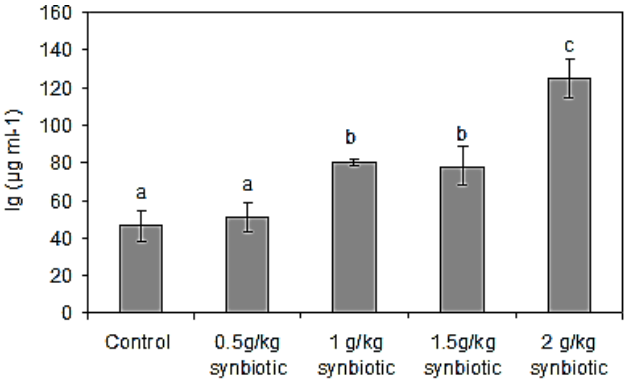

Figure 1. Serum total immunoglobulin (Ig) levels Gibel carp juvenile fed with diets containing different levels of symbiotic. Bars assigned with different superscripts are significantly different (P<0.05).

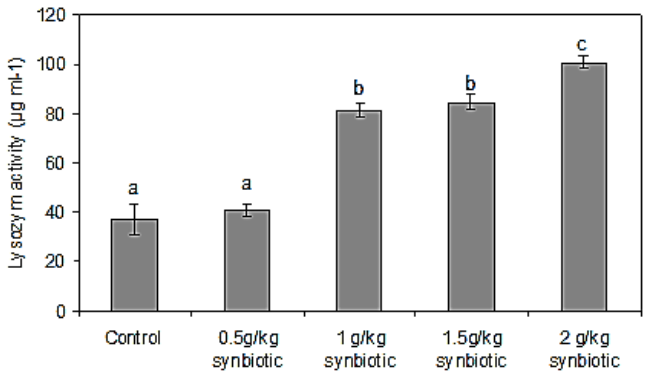

Figure 2. Lysozyme activity of Gibel carp juvenile fed with diets containing different levels of symbiotic. Bars assigned with different superscripts are significantly different (P<0.05)

Data were analyzed by one-way analysis of variance using the statistical software SPSS version 18.0. Subsequent significance between groups was defined by Duncan's multiple range tests. Data are presented as treatment means ± standard of deviation (SD). Differences were considered significant when P value was less than 0.05.

Results

The growth performance of Gibel carp juveniles fed diets supplemented with various levels of dietary synbiotic is presented in Table 1. Compared to the control group, fish fed different dietary levels of synbiotic diet displayed improved (P>0.05) growth performance, including WG, SGR and FCR. There were no significant differences in survival among all treatments (P>0.05) however, fish fed different dietary levels of synbiotic had higher survival compared to the control.

The effects of different dietary levels of synbiotic on the serum total immunoglobulin (Ig) levels and lysozym activity of Gibel carp juveniles are shown in Figures 1 and 2, respectively. Both innate immune responses measured (Ig and lysozyme activity) were significantly higher (P<0.05) in synbiotic fed fish compared to the control group. Fish fed 2.0 g kg^{-1} synbiotic displayed significantly elevated lysozyme activity (100.66 ± 2.51 µg ml^{-1}) and total immunoglobulin (125 ± 10.53 µg ml^{-1}) compared to the control.

Discussion

In the present experiment, the growth performance (P>0.05), total immunoglobulin (Ig) and lysozym activity of Gibel carp were significantly (P<0.05) improved by supplementing the basal diet with synbiotic. This is in agreement with results some studies have revealed the positive effects of synbiotic on the growth performance in fish. Similarly, synergistic effects application of synbiotic was found to enhance the growth performance and survival of White shrimp (*Litopenaeus vannamei*) (Li et al., 2009), Rainbow trout (*Oncorhynchus mykiss*) (Rodriguez-Estrada et al., 2009), European lobster (*Homarus gammarus*) (Daniels et al., 2010), Sea cucumber (*Apostichopus japonicus*) (Zhang et al., 2010), Caspian Kutum (*Rutilus frisii kutum*) fry (Talibi haghighi et al., 2010), Zebrafish (*Danio rerio*)

Larvae (Nekoubin et al., 2012), Rainbow trout (Mehrabi et al., 2012), Grass carp (*Ctenopharyngodon idella*) (Nekoubin and Sudagar, 2012) and Taxas Cichlid (*Herichthys cyanoguttatus*) (Montajami et al., 2012). Improved growth performance may be due to the elevation of digestive enzyme activities, possible improvements of intestine morphology or via synbiotic fermentation by endogenous gut microbes to produce short chain fatty acid (SCFAs). Unlike this study, Ai et al. (2011) reported that no significant interactions were observed between dietary *Bacillus subtilis* and fructooligosaccharide (FOS) on the growth performance, immune response and disease resistance of large yellow croaker (*Larimichthys crocea*). The absence of interactions between FOS and *Bacillus subtilis* in their study was attributed mainly to the absence of the FOS actions. Moreover, the synbiotic effects may be also influenced potentially by the type of species and the environment. Ye et al. (2011) demonstrated that feeding with FOS, MOS or *Bacillus clausii* alone, or in various combinations, yielded best growth performance, feed efficiency and healthy status of the Japanese flounder (*Paralichthys olivaceus*) and, which was more pronounced in fish fed the synbiotics than those fed pre- and probiotics alone. Synbiotics, the combined application of probiotics and prebiotics, is based on the principle of providing a probiont with a competitive advantage over competing endogenous populations; thus, effectively improving the survival and implantation of the live microbial dietary supplement in the gastrointestinal tract of the host (Gibson and Robefroid, 1995). The use of synbiotics it may be possible to produce greater benefits than the application of individual probionts (Merrifield et al., 2010). Stimulation of the immune response of fish through dietary supplements is of high interest for commercial aquaculture (Staykov et al., 2007). In this respect, the innate immune system is very important since aquatic animals are continually vulnerable to numerous opportunistic pathogens and this part of immune response provides the first line of defense for the host (Magnadóttir, 2006). The use of natural immunostimulants is a developing area in aquaculture because they are biodegradable, biocompatible and safe both for the environment and human health (Ortuno et al., 2002).

It is clear from the present study that dietary supplementation of synbiotic can modulate the innate immune responses of Gibel carp. Based on the results, fish fed with 2.0 g kg^{-1} synbiotic had significantly higher total immunoglobulin (Ig) and plasma lysozyme compared to those fed 0.5, 1.0 and 1.5 g kg^{-1} synbiotic and control group. Ye et al. (2011) also reported that lysozyme activity was significantly higher in Japanese flounder (*Paralichthys olivaceus*) fed a synbiotic diet (FOS + Bacillus clausii, MOS+ *Bacillus clausii* or FOS + MOS + *Bacillus clausii*). Another study on white shrimp (*Litopenaeus vannamei*) also reported the synergistic effect between 108 cfu/g *Bacillus megeterium* and 0.2% isomaltooligosaccharides on immune responses and disease resistance (Li et al. 2009). The immunostimulatory nature of synbiotic may be attributed to stimulation of the growth of beneficial bacteria such as lactic acid bacteria (Zhang et al., 2011). Mourino et al. (2012) observed that hybrid surubium (*Pseudoplatystoma* sp.) supplemented with the synbiotic (inulin and *Weissella cibaria*) showed no significant difference in the concentration of serum lysozyme activity (P>0.05), although fish fed the synbiotic had the highest value. In conclusion, growth performance and immunological parameters of Gibel carp (*Carassius auratus gibelio*) juveniles showed positive response to the supplementation of symbiotic for 60 days via diet.

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