

The Influence of Synbiotic on Growth performance and Survival Rate of gray mullet (*Mugilcephalus L.*) juveniles

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Abstract: This study was carried out to evaluate the effect of synbiotic Biomin® IMBO on the growth performance and survival rate of gray mullet (*Mugilcephalus L.*) juveniles (initial weight 6.27 ± 0.021 g). Basal diets were supplemented with 0(control), 0.5, 1.5 and 3.0 g synbiotic per kg-diet in a totally randomized design trial in triplicate group. At the end of the experiment (45 days), the fingerlings showed significant ($P < 0.05$) increases in final weight gain, weight gain (WG %), specific growth rate (SGR) and hepato somatic index (HSI) at all three experimental treatments. The food conversion ratio (FCR) and condition factor (CF) were significantly decreased ($P < 0.05$) in comparison with the control treatment. Furthermore, the synbiotic-fed groups showed significantly higher survival rate compared to the control group ($P < 0.05$). The best results obtained by the fish fed 3.0 g synbiotic per kg-diet. These results reveal that a dietary synbiotic of 3.0 g/kgfed leads to increasing of the growth performance and survival rate as well as improving feeding efficiency in gray mullet juveniles. It seems that the studied synbiotic could serve as a good diet supplement for gray mullet cultures.

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1. Introduction

Synbiotics refer to nutritional supplements that contain both probiotics and prebiotics in a form of synergism, hence synbiotics. When two nutritional ingredients or supplements are given together, the positive results generally follows one of three patterns: additivity, synergism and potentiation. Additive effect occurs when the effect of two ingredients used together approximate to the sum of the individual ingredient effects. In case of synergism, it occurs when the combined effect of the two products is significantly greater than the sum of the effects of each agent administered alone. The term potentiation is used differently, some pharmacologists use potentiation interchangeably with synergism to describe a greater than additive effect and others use it to describe the effect that is only present when two compounds are concurrently (Chou et al., 1991).

Synbiotics affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria and thus improving the host "welfare". In humans, probiotics are mainly active in the small intestine while prebiotics are only effective in the large intestine, so the combination of the two may give a synergistic effect (Gibson and Roberfroid

1995). Actually, The rationale behind their combined use is that it may improve the survival of the probiotic organism, because fermentation can be implemented more effectively as its required specific substrate is readily available, hence, simultaneous presence of live microorganism (i.e. probiotic) and prebiotic reward the host in a proper manner (Collins and Gibson 1999). Although benefits associated with prebiotics and probiotics are desirable, researchers are concerned about a conclusive result, depending on type and amount of pre- and probiotics consumed. Therefore, more studies need to be conducted to provide a better understanding of their direct effects on health (Chitsaz et al., 2016). Limited data is available regarding the application of synbiotics in aquaculture (Ai et al., 2011, Daniels et al., 2010, Rodriguez-Estrada et al., 2009, Zhang et al., 2010). The first application of synbiotics in fish is that of Rodriguez-Estrada et al., (2009). The appropriate use of probiotics in the aquaculture industry were shown to improve intestinal microbial balance and also to improve feed absorption, thus leading to increased growth rate (Fuller 1989, Rengpipat et al., 1998) and also reduced feed conversion ratio (FCR) during the cultural period.

The present study examined the effects of synbiotic Biomin® IMBO on growth performance and survival rate of gray mullet (*Mugilcephalus L.*) juveniles.

2. Materials And Methods

2.1 Rearing conditions and experimental design

Gray mullet juveniles with an average body weight of 6.27 ± 0.021 g were obtained from a well-known farm in south of Iran, transported to the Chabahar Maritime University's laboratory and kept into the aquarium containers (each with a capacity of 50 liters). Fish were acclimatized to laboratory conditions for 2 weeks randomly before being divided into four equal experimental groups (20 fish for each treatment in form of three replicates) representing four nutritional groups as three treatments were conducted to evaluate the effect of symbiotic administered to the gray mullet juveniles and one group without using symbiotic as control group.

2.2 Feeding and symbiotic supplement preparation

The type of symbiotic was applied in this study was Biomin IMBO (Biomin, Herzogenburg, Austria) in which was comprised of probiotic (*Enterococcus faecium* 5×10^{11} CFU/kg) and Fructooligosaccharide (FOS) as prebiotic. To prepare the diets, a commercial pellet diet (containing 41% protein, 17% lipid, 7.3% ash, 14.68 MJ/Kg energy and 96.56% dry matter) was mixed with the supplementation with various levels of symbiotic (0.5, 1.5 and 3.0 g/kg) and water, and made again into pellets (Cerezuela et al., 2008). Then, the feed was air dried under sterile conditions for 4 h and stored at 4 °C until feeding trial. The experimental fish were fed two times daily (9:00 and 17:00) according to 3% of the total biomass of the test fish to apparent satiation for 45 days (Mehrabi et al., 2012).

2.3 Water quality management

Water quality such as, temperature, salinity and pH were monitored daily and measured at 26.79 ± 1.01 °C, 37.42 ± 2.01 ppt, and 1.01 ± 0.19 respectively. The aquaria were daily cleaned by siphoning out the fish feces and uneaten food debris (Mehrabi et al., 2012).

2.4 Determination of growth and survival rate

In order to measure the growth parameters, weight and total length of all fish were measured at every 15 days interval. At the end of the experiment, the fish were fasted for 24 h. Weight gain (WG%), specific growth rate (SGR%), feed Conversion Ratio (FCR), and condition factor (CF%) of gray mullet juveniles were calculated as below (Tacon 1990): $WG(\%) = (W_t - W_i / W_i) \times 100$, $SGR(\%) = (\ln W_t - \ln W_i / D) \times 100$, $FCR = F / W_t - W_i$, $CF(\%) = (W / L^3) \times 100$.

Here W_t and W_i are final and initial body weights (g), L is body length (cm). F is amount of feed intake and D is duration of experimental days. Hepato somatic index was calculated according to the following formula:

$HSI(\%) = \text{liver weight/body weight} \times 100$ (Hung et al., 1993).

The number of dead fish were recorded in every aquarium daily to the end of the experiment, Their survival rate was determined by the according to equation (Hung et al., 1993):

$$\text{Survival rate (\%)} = \frac{N_t - N_0}{N_t} \times 100$$

2.5 Statistical analysis

One-way analysis of variance (ANOVA) was conducted by SPSS software (version 16.0) to determine the significant variation between the treatments. Difference between means was determined and compared by Duncan test. All of the tests used a significance level of $p < 0.05$.

3. Results

The Growth parameters of gray mullet are shown in Table 1. According to the results, treated groups which were fed on a diet contains different levels of symbiotic (0.5, 1.5 and 3 g symbiotic per kg-1 diet) showed significant increasing in weight gain (%), specific growth rate (SGR%) and hepato somatic index (%) in comparison with the control group. The highest average of weight gain was observed in group T3. There was a significant ($p < 0.05$) difference between T1 and control group with T3 but there was not significant ($p < 0.05$) difference between T2 and T3. The addition of symbiotic to the feed also produced the better SGR and HSI with values significantly ($p < 0.05$) higher than the control, more specifically in groups treated with 3% symbiotic. Significant reduction in Feed Conversion Ratio (FCR) in all experiment treatments was clear in comparison with the control group. Best performance condition factor (CF %) was in T3 and it was significantly different with the other treatments.

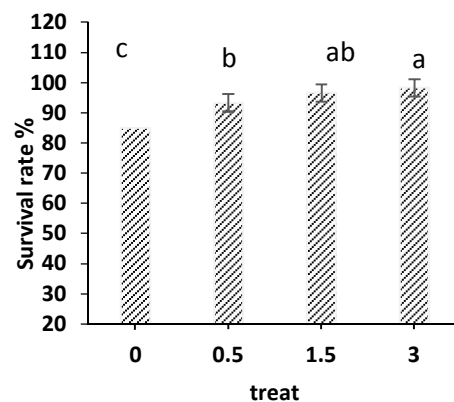


Figure 1. Effect of different levels of symbiotic on survival rate of gray mullet juveniles after 45 day. Different superscript letters denote significant differences ($P < 0.05$) between treatments.

The survival rate of gray mullet is shown in figure 1. According to this figure, in all experiment treatments, survival had significant ($p < 0.05$) increase

in compared to the control group. The highest observed average was in the T3 that was statistically different ($p < 0.05$) with T1 and control groups.

Table 1. Effect of feed supplementation with synbiotic on growth performance in gray mullet juveniles after 45 days.

Growth Indices	Control	T1with 0.5 g/kg	T2with 1 g/kg	T3with 1.5 g/kg
Initial weight (g)	6.27 ± 0.02^a	6.27 ± 0.02^a	6.27 ± 0.02^a	6.28 ± 0.01^a
Final weight (g)	7.52 ± 0.01^d	9.20 ± 0.01^c	9.88 ± 0.06^b	10.21 ± 0.01^a
weight gain (%) (g)	14.02 ± 4.40^c	31.77 ± 14.37^b	38.21 ± 18.20^{ab}	42.72 ± 19.23^a
SGR (%)	0.29 ± 0.08^c	0.59 ± 0.24^b	0.69 ± 0.30^b	0.77 ± 0.31^a
FCR	4.97 ± 1.44^a	2.70 ± 1.64^b	2.33 ± 1.50^b	2.00 ± 1.21^c
CF (%)	1.01 ± 0.05^a	1.00 ± 0.06^a	0.97 ± 0.08^a	0.92 ± 0.10^b
HSI (%)	1.85 ± 0.06^c	1.86 ± 0.06^c	2.06 ± 0.21^b	2.45 ± 0.61^a

Groups with different alphabetic superscripts differ significantly at $p < 0.05$ (ANOVA).

4. Discussion

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 Roberfroid 1995). The use of synbiotics may possibly produce greater benefits than the application of individual probionts (Merrifield et al., 2010). Moreover, the synbiotic effects may be also influenced potentially by the type of species and the environment (Mahghani et al., 2014). In this study, the fed gray mullet juveniles diets containing synbiotic significantly showed higher final weight gain, WG%, SGR, HSI and survival. The best FCR and CF values were observed with synbiotic supplemented diets ($p < 0.05$). So, it suggests that addition of synbiotic improved feed utilisation by the gray mullet juveniles. The food conversion ratio (FCR) in the experimental treatments was significantly decreased in comparison with control treatment ($p < 0.05$) the lowest was detected with a dietary synbiotic of 3.0 g kg⁻¹. Food conversion ratio is considered to be one of the economic benefits of aquaculture because, in addition to reduction in feeding costs due to decreased feeding, it prevents deteriorating of the cultivation media and degradation of water quality eventually leading to increased profits (Falahatkar, Soltani, Abtahi, Kalbassi, Pourkazemi & Yasemi 2006). This may suggest that gray mullet is able to utilize food efficiently while receiving relatively small amount of synbiotic, which, in turn, this is an advantage. These results are in agreement with results of Mehrabi et al., (2012) that he reported all treatments supplemented with symbiotic showed significant ($p < 0.05$) increase

in final mean weight, WG(%), SGR, CF, FCE and survival rate, compared to the control group. Similar finding were observed by Firouzabakhsh et al., (2014) that in rainbow trout (*Oncorhynchus mykiss*) showed significant ($P < 0.05$) increases in final mean weights and SGR at all three experimental treatments. In their study, the best feed conversion ratio (FCR), feed conversion efficiency (FCE) and maximum survival rate were also obtained by the fish fed 1.0 g synbiotic kg⁻¹ diet. Application of synbiotics was found to enhance the growth performance and survival of European lobster (*Homarus gammarus*) (Daniels et al., 2010), Zebra fish (*Danio rerio*) larvae and Caspian roach (*Rutilus rutilus*) (Chitsaz et al., 2016), too. YE et al., (2011) demonstrated that feeding with FOS, MOS or *Bacillus clausii* alone, or in various combinations, yielded best growth performance (Special weight gain), feed efficiency and healthy status of the Japanese flounder (*Paralichthys olivaceus*), which was more pronounced in fish fed the synbiotics than those fed pre- and probiotics alone. In research on Texas Cichlid (*Herichthys cyanoguttatus*) Larvae (Montajami et al., 2012), common carp (*Cyprinus carpio*) fingerlings (Dehaghani et al., 2015), Grass Carp (*Ctenopharyngodon idella*) (Nekoubin et al., 2012) and Gibel carp (*Carassius auratus gibelio*) juveniles (Mahghani et al., 2014) the growth performance were significantly ($p < 0.05$) increased by supplementing the basal diet with synbiotics but, survival rate had not showed any significant difference among treatments. Unlike this study, Ai et al., (2011) reported that in their study no significant interactions were observed between dietary *Bacillus subtilis* and fructooligosaccharide (FOS) on the specific growth rate (SGR), hepato somatic index (HSI) and survival rate of large yellow croaker (*Larimichthys crocea*). The absence of interactions between FOS and *Bacillus*

subtilis was attributed mainly to the absence of the FOS actions. In this study, the synbiotic-fed fingerlings showed higher survival rate ($P < 0.05$) at all treatments compared with those from the control (Figure1). It is in agreement with the results reported by Zhang et al., 2010. They reported the animals (sea cucumber) fed with diets supplemented with 0.25% and 0.50% FOS at each *B. subtilis* level had notably lower cumulative mortality after 14 days following *V. splendidus* exposure ($P < 0.05$). Rodriguez-Estrada et al., (2009) stated that application of dietary *Enterococcus faecalis* and MOS provided a wide range of benefits regards to immune response and survival in challenge with *V. anguillarum*. However, they noted that synbiotic feeding (*E. faecalis* + MOS) yielded significantly better results than either individual probiotic or prebiotic application compared to the control group.

Accordingly, data in this study showed that addition of synbiotic (3 g/kg) to the gray mullet feed caused the best growth performance and survival rate with values statistically higher than the values of the control group. All and all, it can be concluded that addition of synbiotic in feeding regime could increase the growth performances, survival rate and improve feeding efficiency in gray mullet; hence it is recommended to prioritize the dietary manipulation for growth and health management in aquaculture research.

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