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Effects of dietary prebiotic MHF-Y and starvation on the compensatory growth, survival, and some hematological parameters in common carp *Cyprinus carpio* fry

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Abstract

This study aimed to determine the effects of dietary prebiotic Mito (MHF-Y) on compensatory growth common carp *Cyprinus carpio* fry (n= 300; 4.5 ± 0.05 g) following one week starvation. The experiment was conducted within 60 days with three triplicate treatments: a control fed with non-prebiotic diet with no starvation period (T1), a 2nd group starved for one week then fed 2.0 g of prebiotic per kg diet (T2), and the 3rd one received no prebiotic following one week starvation (T3). The results showed the highest final weight and length in the control followed by T2 ($P< 0.05$). T1 and T2 gained the greatest (0.81 ± 0.00 and 0.57 ± 0.01 d^{-1}, respectively) specific growth rate (SGR). The condition factor significantly increased (1.8 ± 0.11) in T3 while T1 and T2 were not different in this respect ($P > 0.05$). The lowest (2.26 ± 0.02 and 2.59 ± 0.1) feed conversion ratio (FCR) and highest (1.2 ± 0.04 and 1.38 ± 0.01) protein efficiency ratio (PER) were recorded in T1 and T2, respectively. Numbers of red blood cells were similar in the three treatments ($P > 0.05$). Hemoglobin and hematocrit were utmost in T3 and T1, respectively. T2 contained the highest (13800 ± 600) number of white blood cells (WBC). The treatments were not different in WBC differential counts of basophils, eosinophils, and monocytes. The greatest (77.59 ± 1.00) and lowest (74.33 ± 0.57) amounts of lymphocytes, respectively, were detected in T2 and T1. The results show that the addition of 2% of prebiotic Mito in the diet of carp fry could not improve their growth performance, it, however, could give rise to better results compared with T3 that fed no prebiotic after starvation; in addition, the fry in T2 showed the best results concerning improved immunity.

Key words: Common carp, Prebiotic Mito, Blood factors, Compensatory growth.

Introduction

The common carp, *Cyprinus carpio*, accounts for one of the most important commercial fish in this family (Vosoughi and Mostajir, 2001). Due to the fact that a considerable amount of the culture costs is dedicated to nutrition, major challenges in commercial aquaculture of this species are improvements in formulated diets for growth optimization and health promotion. One way to deal with such a challenge is to use food supplements such as probiotics, prebiotic, and synbiotics, which, in addition to growth promotion, have beneficial effects on the host immune system (Hoseinifar *et al*., 2011).

Prebiotics are non-digestible foodstuff that stimulate growth through activation of one or a limited number of intestinal bacteria leading to beneficial effects to the host and its health improvement (Hanley *et al*., 1995). The prebiotic Mito (MHF-Y) known in Japan as an animal feed mixed with sugar extract, contains 1 to 4% dextran granular powder. Dextran is a component of glucose produced from sugar fermentation. This sugar is obtained from binding of 1 & 6-α-glycosides, while starch and cellulose are achieved by binding 1 & 4-α or β-1 & 4- glycosides. Since the binding between 1 and 6 is the most resistant to acidic degradation, the digestion of dextran would be difficult by the gastric secretions in animals. This polymer is broken to smaller dextran molecules called isomalto oligosaccharides (LMD) in the course of stomach to the intestine by specific enzymes in the intestinal mucosa. The residue of LMD is used as a food source for enteric bacteria; it also accelerates the growth of beneficial lactic acid bacteria such as *Lactobasillus* and *Bifidus*, which inhibit the growth of bacteria *E .coli* and *Salmonela* through the production of lactic acid resulting in decreased intestinal pH.

Among the few studies conducted to date on the use of prebiotic in the diet of farmed aquatics, nearly all researchers have focused on a few specific compounds (including inulin and mannan oligosaccharides or MOS) (Gence *et al*., 2007a,b; Dimitroglou *et al*., 2009; Akrami *et al*., 2012). Therefore, this research tried to use a new commercial prebiotic in carp diet in order to introduce the compound to aquaculture industry. The aim of this study was, therefore, to evaluate the effects of different levels of prebiotic Mito on the compensatory growth, and some hematological and nutritional parameters following one week starvation in the common carp fry as one of the most important farmed fish in Iran.

Materials and methods

The fry of *C. carpio* (n= 300; 4.25 ± 0.04 g) were obtained from a local hatchery in Nasr Fish Propagation Center located in the north of Iran and transferred to the experiment place. After the initial adaptation to the new temperature and test diet for two weeks, a total of 270 fry were biometried for the length (63.16 ± 0.2 mm) and weight (4.5 ± 0.05 g) measurements, and randomly stocked in each tank. This experiment was designed as completely randomized including three triplicate treatments (n= 30 per replicate). The treatments were: a control fed with non-prebiotic diet with no starvation period (T1), a 2nd group starved for one week then fed 2.0 g of prebiotic per kg diet (Nikbakhsh, 2013) named as T2, and a 3rd one received no prebiotic following one week starvation (T3). The food used in this experiment was the pellets for carp fry (FFT). The prebiotic diet was prepared by the addition of 2 g prebiotic MHF-Y (Mito Corp., Japan) to kg diet. Analysis of compounds in the diet used in this study is shown in Table 1.

Table 1. Analysis of compounds in the experimental diet.

Compound	Content (%)

Crude protein	32.23
Crude fat	5.5
Ash	8.00
Fiber	3
Fiber	0.8

During the experimental period, the carp fry were fed based on a percentage of body weight (4%) within three times (8, 13 and 18 hrs). All physicochemical conditions (such as temperature, oxygen content, pH, etc.) of water in the tanks were monitored daily during the experiment and maintained within optimum levels. At the completion of the experiment (60 days), the fry were not fed for 24 hours. Afterward, all fish were caught and weighed. Then four fry per replicate were randomly sampled, anesthetized by clove extract solution (1.0 g L^{-1}; Mohammadi *et al*., 2001); then blood samples were taken from their caudal fins, transferred to heparinated tubes, and stored at -4 °C. Red and white blood cells (RBC & WBC) were counted using a Neubauer hemocytometer (Stoskopf, 1993), hematocrit was assessed through microhematocrit method (Rehulka *et al*., 2011), and hemoglobin level was evaluated by a kit and spectrophotometry (540 nm) (Blaxhall and Daisley, 1973). To calculate the growth and nutritional parameters, the following formula were applied:

Weight gain= Initial weight (g) - weight (g) (Tacon, 1995)

Specific growth rate (SGR, % per day) = 100 × [(Ln final weight - Ln initial weight)/ experimental period] (Hevroy *et al*., 2005)

Condition factor (CF) = 100 × [weight of fish (g)/ (length in cm)^3] (Ai *et al*., 2006)

Protein efficiency ratio (PER) = Weight gain (g)/protein consumed (g) (Helland *et al*., 1996)

Feed conversion ratio (FCR) = fish weight (g) / ingested food (g) (Hevroy *et al*., 2005)

Survival rate (%) = 100 × (Initial number of fish – Final number of fish) (Ai *et al*., 2006)

In order to analyze the data, first the normality test was performed by Shapiro-Wilk test. The data on changes in growth, nutritional and blood factors of fish were analyzed through one-way analysis of variance (ANOVA). Differences between the treatments were compared by Duncan's multiple range test. Raw data were first processed in Microsoft Excel 2010 and the presence or absence of a significant difference was verified at a confidence level of 5% using SPSS (version 21).

Results

The highest weight gain (8.84 ± 0.09 g) was recorded in T1 (control) and the lowest (3.7 ± 0.18 g) in T3 (Table 2). T1 and T3, respectively, were the longest (96.63 ± 0.47 mm) and shortest (75.5 ± 1.40 mm) fry ($P < 0.05$).

Table 2. Growth indices (value ± standard deviation) at different treatments measured in carp fry fed Mito prebiotic

Index \ Treatment	Control (T1)	MHF-Y (T2)	Basal diet (T3)
Final weight (g)	13.10±0.13^c	9.38±0.20^b	8.03±0.15^a
Final length (mm)	96.63±0.47^c	83.90±0.36^b	75.50±1.40^a
Body weight gain (g)	8.84±0.09^c	5.14±0.21^b	3.70±0.18^a

% Body weight gain	207.99±0.60^{c}	121.31±5.00^{b}	88.53±0.15^{a}
SGR (% day^{-1})	0.81±0.00^{c}	0.57±0.01^{b}	0.46±0.01^{a}
Condition factor	1.44±0.00^{a}	1.50±0.00^{a}	1.80±0.11^{b}

*Values with similar superscript letters are not statistically different (P > 0.05).

The fry of carp in T1 received a basal diet with no starvation showed the highest values (0.81 ± 0.00) of SGR while T3 fed prebiotic attained the smallest SGRs (0.46 ± 0.01). CF significantly increased (1.8 ± 0.11) in T3 while T1 and T2 with lower CF values were not different in this respect (P > 0.05).

Significant differences (P < 0. 05) were observed among some treatments in FCR and PER (Table 3). The fry in T1 displayed the lowest FCR value (2.26 ± 0.02) whereas the highest value (3.18 ± 0.14) was found in T3. The PER of fish in T1 was significantly highest (1.38 ± 0.01) in comparison with the lowest one in T3 (0.98 ± 0.04).

Table 3. Nutritional parameters (value ± standard deviation) measured in the treatments

Index \ Treatment	Control (T1)	MHF-Y (T2)	Basal diet (T3)
Feed conversion ratio	2.26±0.02^{a}	2.59±0.10^{b}	3.18±0.14^{c}
Protein efficiency ratio	1.38±0.01^{c}	1.20±0.04^{b}	0.98±0.04^{a}

According to Table 4, the number of RBC in fish showed no significant differences between the control (T1) and the other groups (P > 0.05), though T1 contained the greatest (111,0000 ± 36055 mm^{-3}) RBC numbers. Hemoglobin was not different between T2 and T3 but T1 significantly differed from those detected in T2 T3 (P< 0.05). T1 also had the utmost level (9.91 ± 0.05) of hemoglobin. Hematocrit was almost similar in T2 and T3 and both groups were statistically different from T1 with the highest (32.66 ± 4.6) hematocrit estimate.

Table 4. Blood parameters (value ± standard deviation) at different treatments measured in carp fingerling fed Mito prebiotic

Blood factors \ Treatment	Control (T1)	MHF-Y (T2)	Basal diet (T3)
RBC (per mm^{3})	1110000±36055^{a}	10336700±58862^{a}	1090000±69282^{a}
Hemoglobin (g/dl)	9.91±0.05^{b}	9.78±0.03^{a}	9.80±0.05^{a}
Hematocrit (%)	32.66±4.60^{b}	24.00±1.00^{a}	25.00±1.00^{a}
WBC (per mm^{3})	10200±600^{a}	13800±600^{b}	100033±929^{a}
Basophil (%)	1.00±0.10^{a}	0.66±0.57^{a}	0.66±0.70^{a}
Eosinophil (%)	3.00±1.00^{a}	2.33±0.57^{a}	2.66±0.57^{a}
Neutrophil (%)	20.66±0.57^{b}	19.33±0.57^{a}	20.33±0.57^{ab}
Monocyte (%)	1.00±0.10^{a}	0.66±0.50^{a}	1.00±0.10^{a}
Lymphocyte (%)	74.33±0.57^{a}	77.59±1.00^{b}	75.33±0.57^{a}

*Values with similar superscript letters are not statistically different (P > 0.05).

The WBC count markedly rose (13800 ± 600) in T2 being dissimilar with T1 and T3. Differential WBC count revealed no statistical dissimilarities between the treated groups in the amounts of basophils, eosinophils, and monocytes ($P > 0.05$). The percentages of neutrophil was markedly different among the treatments with the highest (20.66 ± 0.57) and lowest (19.33 ± 0.57) numbers in T1 and T2, respectively. The values of lymphocytes were greatest (77.59 ± 1.00) in T2 and the least in T1 (74.33 ± 0.57), but T3 and T1 were not significantly different.

Discussion and conclusion

The results of assessing the addition of prebiotic MHF-Y and starvation period on compensatory growth of *C. carpio* fry indicate that feeding 2 g/kg of prebiotic in diet to fish starved for one week leads to significant improvements in final weight gain, percentage of body weight increase, final length, and SGR, all of which highly increased in T1 (control) but markedly decreased in T3. Most of fish spend long or short periods of starvation throughout their lives. The responses to starvation periods are different in various species of fish. During the period of feeding limitation, fish consumes the nutrients reserves of body. At the time of re-feeding, the phenomenon of compensatory growth is activated and increases growth rate depending on fish species, age, starvation duration, and food type at re-feeding time (Heide et al., 2006).

It was reported in rainbow trout *Oncorhynchus mykiss* that although starvation and re-feeding periods strongly reduced enzymatic activities of trypsin, chymotrypsin, and lipase at the end of feeding deprivation, the enzymatic activities of starved treatments reached those in the control, and the fish were able to recover their digestive capacity following food deprivation (Imani and Iranparast 2008). Emadi Sheibani *et al*. (2013) studied the effects of starvation and re-feeding periods on the hepatic tissue of *Salmo trutta caspius* fry from the Caspian Sea and found that food deprivation resulted in the consumption of hepatic reserves leading to tissue damage, but the liver was able to repair and compensate for the damage caused by starvation when the fish were re-fed and could recover energy stores. Abolfathi (2009) examined the effects of starvation and re-feeding on roach (*Rutilis rutilus caspicus*) and detected that fish growth after the starvation period was compensable, which corresponds with the results of this study. Further study on the roach by Taheri and Aliasghari (2012) revealed that control group showed the highest values in weight gain, daily growth rate, and SGR but the lowest CF, which correspond to what found here. In a study on Atlantic cod (*Gadus morhua*), compensatory growth could fully offset the weight loss due to starvation period with significant differences between the final weight of the treated and control groups after starvation (Jobling *et al*., 1994). Similar results were recorded in the pond fish (*Carassius auratus gibelio*) (Xie *et al*., 2001).

In most cases where the re-feeding was able to completely compensate the decreased growth, there were long periods of starvation and compensatory growth. Studies conducted the Cyprinid species indicate that the process of compensatory growth appears 6 to 12 days after re-feeding (Wieser *et al*., 1992) and in most cases, complete recovery of lost growth takes 2 to 4 weeks, which is also affected by environmental factors such as temperature (Ali *et al*., 2003). Hence, by increasing the re-feeding duration, compensatory growth phenomenon is likely to further strengthen. It can, therefore, be stated that short-term starvation

and re-feeding periods cannot provide the necessary conditions for compensatory growth. The reason for the lower growth rate of T2 compared to the control group in this study could also be related to this topic.

Survival is one of the important parameters in aquaculture and can be influenced by hunger period. In most cases, fish starved for a short period display high survival rates, but if the hunger period is prolonged, mortality increases. In the current study, no significant differences were observed in survival rates between the control and the other treatments all showing complete survival, which could be due to the short periods of starvation applied. Similarly, the study by Taheri and Ali (2012) on roach reported no significant differences in survival of fish, which correspond with the results of this study.

The estimated FCR showed significant differences between the treatments with the smallest and greatest values in T1 (control) and T3, respectively. The PER was also significantly different among the three treatments with the highest in control and lowest in T3. Nickbakhsh (2013) examined prebiotic Mito in common carp (*Cyprinus carpio*) and detected that from levels of 1, 2 and 3 g k^{-1} of dietary prebiotic, only 2 g k^{-1} of Mito-based diet led to significant PER differences compared to the control group. PER was utmost with 2 g k^{-1} of prebiotic and lowest in control.

One of the most reliable indicators of health status and fish physiology is measurement of blood parameters that is affected by nutrition, environmental factors, age, sex and other physiological parameters (Gazorani Farahani, 2008). In this study, changes in RBC in common carp fry experienced the effect of prebiotic Mito as well as hunger periods did not show significant differences between the three groups, but the greatest and lowest amounts were measured in control and T2, respectively. Increased hemoglobin concentration affect the transmissibility of respiratory gases in blood, the efficiency of the heart, and fish weight gain (Gazorani Farahani, 2009). The amount of hemoglobin in the control group showed significant differences with the other treatments, which shows the superiority of respiratory status in the control fry. Hematocrit is an important and useful yet simple and quick indicator for blood assessment. In the present study, the highest hematocrit was observed in T1 as well. Hisano *et al*. (2007) reported that consumption of at least 2% of dehydrated yeast (the main source of mannan oligosaccharide or MOS) in the diet of tilapia (*Oreocheromis niloticus*) had no effects on hematological parameters, which corroborates our results. On the other hand, Welker *et al*. (2007) examined the effect of probiotic MOS on the catfish (*Ictahurus punctatus*) and reported that the blood parameters of the fish fed dietary MOS were not different from the control contrary to the results of this study.

The number of WBC and its components such as lymphocytes, neutrophils, and monocytes are important indicators of fish health and one of the main parts of the body's non-specific immune system (Ahmadifar *et al*., 2009). Most of WBC and lymphocytes as immune defense were observed in T2 of this study, though T1 and T3 were not significantly different. This represents an increase of stimulating the immune system in fish fed with prebiotic Mito. It seems that prebiotic Mito increasingly stimulates and boosts the immune system of fish as a result of antimicrobial activity against pathogens and also an impact on the immune response by increasing the number of WBC. Sado *et al*. (2008) showed that farmed young tilapia (*O. niloticus*) fed prebiotics MOS at 0.2, 0.4, 0.6, 0.8, and 1.0 percent did not exhibit increased levels of leukocytes and also significant differences in blood parameters compared with the control, which do not agree with our finding. Razeghi Mansour *et al*. (2012) observed no significant effects of dietary MOS (2 and 4 g k^{-1}) on the hematology of juvenile beluga *Huso huso*, which is not in agreement with our observations. Andrews *et al*. (2009) through addition of probiotic MOS (1, 2 and 4 percent) in the diet of

Labeo rohita fingerlings noticed significant rises in the WBC, RBC, hemoglobin, serum protein, albumin, and globulin in the treated fish compared to the control.

Stimulating the immune system can cause improved resistance of aquatic organisms, and under unfavorable environmental conditions likely associated with certain stresses such as chemical, physical, and infectious tensions, become effective and ultimately increase production efficiency (Ahmadifar *et al*., 2009). In this study, basophils, eosinophils, and monocytes did not show significant differences in the three treatments relative to each other. Del Rio-Zaragoza *et al*. (2011) used beta-glucan (0.5, 0.1, and 0.5 percent) in the diet of red snapper (*Lutjanus guttatus*) for 5 weeks and reported that levels of 0.5 and 0.1 significantly increased monocytes and neutrophils in the 2nd and 4th weeks in comparison to other treatments, which is contrary to the results of this study.

Altogether, according to the results of this study, it can be concluded that, although the use of prebiotic Mito in the diet largely caused compensatory growth in common carp fry, but the examined short period of starvation and consequent compensatory growth was not so sufficient that exceed those of the control group with the highest growth rate. However, the hematological results show that the addition of 2% probiotic Mito in the diet significantly elevates immune status of the fry compared to the control group, which can be used as a recommended suitable complement to the diet.

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