Effects of dietary prebiotic Mito (MHF-Y) and starvation on the compensatory growth, survival, and hematological parameters in Common carp *(Cyprinus carpio* L, 1758)

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Received: November 2017 **Accepted:** April 2018

[DOR: 20.1001.1.2345315.2018.4.1.2.6]

[DOI: 10.29252/ijaah.4.1.82]

Abstract

In order to study the effect of prebiotic Mito on compensatory growth following one week starvation in common carp, the fish (4.5 ± 0.05 g) were examined for 60 days in three treatments. A control fed with non-prebiotic diet with no starvation (T1), a 2nd group starved for one week then fed 0.2% of prebiotic (T2) and 3rd group received no prebiotic diet after one week starvation. The highest percent of weight gain and specific growth rate and condition factor were obtained in the control followed by T2 with significant differences (P<0.05). The lowest amount of food conversion ratio and the highest amount of protein efficiency ratio were recorded in T1, too. The mortality rate was zero in all treatments. The number of red blood cells showed insignificant differences between the control and the other groups (P> 0.05). Hemoglobin and hematocrit values significantly differed between T1 and two other treatments. T2 contained the highest number of white blood cells and lymphocyte while the percent of basophil, eosinophil and monocyte did not differ between treatments significantly (P> 0.05). So, although the use of 0.2% prebiotic largely caused compensatory growth in carp but was not so sufficient that exceed those of the control group. However, the addition of prebiotic Mito significantly elevated hematological status of the fish.

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Keywords: Common carp, Prebiotic Mito, Hematological parameters, Compensatory growth, Survival

Introduction

The Common carp (*Cyprinus carpio* Linnaeus, 1758) accounts for one of the most important commercial fish in this family. 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 formulating 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, Mirvaghefi, Mojazi Amiri, Khoshbavar Rostami, Poor Amini & Darvish Bastami 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, Brown & Carbery 1995). The prebiotic Mito (MHF-Y) known in Japan as a food supplement mixed with sugar extract, contains 1 to 4% dextran granular powder. Dextran is a component of glucose produced from sugar fermentation and the digestion of dextran would be difficult by the gastric secretions (Hoseinifar *et al.* 2011).

Compensatory growth in fish is a phase of fast growth, which occurs after the re-feeding of fish following a period of feed deprivation or after abnormal conditions such as low temperature. Compensatory growth may be divided into different categories according to growth and feeding performance including full compensation, partial compensation, and overcompensation. Fish subjected to feed deprivation may partially or completely catch up in body size that have not undergone feed deprivation and is fed continually that in some studies on European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) (Türkmen, Eroldoğan, Yılmaz, Ölçülü, Inan, Erçen & Tekelioğlu 2012) and juvenile sobaity (*Sparidentex hasta* Valenciennes, 1830) have been demonstrated (Torfi, Marammazi, Yaghoubi, Yavari, Agh & Gisbert 2017). Fish even maybe catch up to more size of those fed continuously (over compensation), which have been demonstrated on hybrid sunfish (*Lepomis macrochirus* Rafinesque, 1810 × *L. gibbosus* Linnaeus, 1758) (Hayward, Noltie & Wang 1997).

Effect of starvation and re-feeding on growth of Common carp and other species was studied several times in different feeding schedule (Prabhakar, Sardar & Dos 2008; Assareh, Mohammad Nejad, Faghani & Karbakhsh 2012; Pang, Fu, Li & Zhang 2016; Kondera, Kosciuszko, Dmowska & Witeska 2017), but there is no report about effect of one prebiotic on compensation growth in fish. Also, among the studies conducted on the use of prebiotic in the diet on the growth of farmed species, nearly all researchers have focused on a few specific compounds (including inulin and mannan oligosaccharides or MOS) (Genc, Aktas, Genc & Yilmaz 2007; Dimitroglou, Merrifield, Moate, Davies, Spring, Sweetman & Bradley 2009; Akrami, Razeghi-Mansour, Chitsaz, Ziaee & Ahmadi 2012). The only report about using prebiotic Mito in the fish diet, is Nikbakhsh and Bahrekaemi study (2017) which examined it in Common carp food. Therefore, this research tried to use this new commercial prebiotic after one week starvation in carp diet to examine the effect of Mito on compensation growth and hematological parameters.

[DOR: 20.1001.1.2345315.2018.4.1.2.6]
[DOI: 10.29252/ijaah.4.1.82]

Materials and Methods

Providing of samples and experimental procedure

The 300 fish of *Cyprinus carpio* were obtained from a local hatchery in Nasr Fish Propagation Center located in the north of Iran and transferred to the experimental place. After the initial adaptation to the new temperature and test diet for two weeks, a total of 270 fish were measured for the length (63.16 ± 0.2 mm) and weight (4.5 ± 0.05 g) and randomly stocked in each aquarium (1.5 × 0.8 × 0.5 m, 0.6 m^3). 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 gr of prebiotic per kg diet (based on the result of Nikbakhsh & Bahrekazemi 2017) named as T2, and a 3rd group received no prebiotic diet following one week starvation (T3).

Providing of experimental diets

The elements of the food and the analysis of compounds in the diet used in this study (Abzian Shomal Co. Babol, Iran) is shown in Table 1. The prebiotic diet was prepared by the addition of 2 gr prebiotic MHF-Y (Mito Corp., Japan) to kg of diet and after the even distribution of prebiotic in the diet, the pellets became by a meat grinder (size: 2 mm). The pellets were dried in an oven (Pars Azma - Iran) at 55 ºC for 24 hours and were stored at 4 °C in the refrigerator (Akrami, Ghelichi & Zareie 2011). The basal diet, which used for feeding of other treatments was prepared based on the same method (without adding the prebiotic) too.

During the experimental period (60 days), the fish were feeding based on a percentage of body weight (4%) within three times (8, 13 and 18 hr). All physicochemical conditions (such as temperature, oxygen content, pH, etc.) of water in the aquarium were monitored daily during the experiment and maintained within optimum levels (Table 2).

Table 1. Analysis of elements and compounds in the experimental diet

Ingredients	Per cent
fish meal (57.35% protein)	25
gluten	10
wheat flour	20
canola oil	8
soybean meal	24
meat meal	10
mineral premix*	1.5
*vitamin premix	1.5
Anti-fungi	0.1
stable vitamin C	0.13
binder (Molasses)	0.2
Proximate composition	%
Crude protein	32.23
Crude lipid	5.5
Moisture	11.95
Ash	6

*Mineral premix: Mg, Fe, Zn, Cu, I, Se, and Choline Chloride.

* Vitamin premix: E, A, D3, B1, B2, B5, B6, B12, and K.

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Table 2. Conditions of water during the study period

Parameter	Value	Optimal range
Water temperature (°C)	21-23	25-17
pH	7.7-8.5	6.5-8
Ammonia (mg l^{-1})	0.6	0-0.02
Nitrite (mg l^{-1})	0.0	0-0.1
Total hardness (mg l^{-1})	285	10-400
Dissolved oxygen (mg l^{-1})	8	5-10

Growth and nutritional parameter measurements

At the end of the experiment (60 days), the fish were not fed for 24 hours. Afterward, the weigh and length of all fish was measured. To calculate the growth and nutritional parameters, the following formula were applied (Helland, Grisdale & Nerland 1996; Ai, Mai, Tan, Xu, Duan, Ma & Zhang 2006):

Weight gain (g) = Final weight (g) - Initial weight (g)

Weight gain (%) = (Final weight (g) - Initial weight (g)) ×100

Total length (mm) = Final length (mm) – Initial weight (mm)

Specific growth rate (SGR, % per day) = 100 × [(Ln final weight - Ln initial weight)/ experimental period]

Condition factor (CF, %) = 100 × [weight of fish (g)/ (length in cm)^3]

Protein efficiency ratio (PER) = Weight gain (g)/protein consumed (g)

Feed conversion ratio (FCR) = fish weight (g) / ingested food (g)

Mortality rate (%) = 100 × (Initial number of fish – Final number of fish)

Hematological analysis

At the end of the study, four fish per replicate were sampled and anesthetized by clove extract solution (1.0 g L^{-1}), (Nikbakhsh & Bahrekazemi 2017). After anesthesia, the blood samples were taken from fish caudal fins, then 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, Minarik, Cink & Zalak 2011), and hemoglobin level was evaluated by a kit and spectrophotometry (540 nm) (Blaxhall & Daisley 1973). Also, the white blood cells differential count was done via preparation of blood film and Giemsa staining method (Stoskopf 1993).

Statistical analyses

In order to analyze the data, first the normality test was performed by Shapiro-Wilk test. The data were analyzed through one-way analysis of variance (ANOVA). Differences between the treatments were compared by Duncan's multiple range test. The 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).

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Results

Growth performance and survival

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 3). T1 and T3, respectively, were the longest (96.63 ± 0.47 mm) and shortest (75.5 ± 1.40 mm) fish ($P < 0.05$). The fish in T1 received a basal diet with no starvation showed the highest values of SGR while T3 attained the smallest SGR amount (Table 3). CF significantly increased in T1 while T2 and T3 with lower CF values were not different in this respect ($P > 0.05$). Also, the mortality rate was zero in all treatments.

Table 3. Growth indices (mean ± SD) at different treatments measured in fish fed Mito prebiotic

Treatment Index	Control (T1)	MHF-Y (T2)	Basal diet (T3)
Final weight (g)	13.10 ± 0.13^a	9.38 ± 0.20^b	8.03 ± 0.15^c
Final length (mm)	96.63 ± 0.47^a	83.90 ± 0.36^b	75.50 ± 1.40^c
Body weight gain (g)	8.84 ± 0.09^a	5.14 ± 0.21^b	3.70 ± 0.18^c
Body weight gain (%)	207.99 ± 0.60^a	121.31 ± 5.00^b	88.53 ± 0.15^c
Specific growth rate (% day^{-1})	0.81 ± 0.00^a	0.57 ± 0.01^b	0.46 ± 0.01^c
Condition factor (%)	1.80 ± 0.11^a	1.50 ± 0.00^b	1.44 ± 0.00^b

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

Nutritional parameters

Significant differences were observed among some treatments in FCR and PER ($P < 0.05$) (Table 4). The fish in T1 displayed the lowest FCR value, whereas the highest value 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.

Table 4. Nutritional parameters (mean ± SD) at different treatments measured in fish fed Mito prebiotic

Treatment Index	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^a	1.20 ± 0.04^b	0.98 ± 0.04^c

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

Hematological parameters

According to Table 5, 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 RBC numbers. Hemoglobin was not different between T2 and T3 but, T1 significantly differed from those detected in T2, and T3 ($P < 0.05$). T1 also had the utmost level of hemoglobin. Hematocrit was almost similar in T2 and T3 and both groups were statistically different from T1 to the highest hematocrit estimate.

The WBC count markedly rose in T2 in compared to T1 and T3 treatments. Differential WBC count revealed no statistical difference between the treated groups in the amounts of basophils, eosinophils, and monocytes ($P > 0.05$).

[DOR: 20.1001.1.2345315.2018.4.1.2.6]
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The percentages of neutrophil were markedly different among the treatments with the highest and lowest numbers in T1 and T2, respectively. The values of lymphocytes were greatest in T2 and the least in T1, but T3 and T1 were not significantly different (Table 5).

Table 5. Blood parameters (mean ± SD) at different treatments measured in fish fed Mito prebiotic

Treatment Blood factors	Control (T1)	MHF-Y (T2)	Basal diet (T3)
RBC (N $(mm^3)^{-1}$	1110000 ± 36055[a]	10336700 ± 58862[a]	1090000 ± 69282[a]
Hemoglobin (g dl^{-1})	9.91 ± 0.05[a]	9.88 ± 0.03[b]	9.80 ± 0.05[b]
Hematocrit (%)	32.66 ± 4.60[a]	25.00 ± 1.00[b]	24.00 ± 1.00[b]
WBC (N $(mm^3)^{-1}$	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[a]	19.33 ± 0.57[b]	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)

Discussion

The results of this study indicated that feeding 2 g of prebiotic in kg of diet to fish starved for one week, leads to significant improvements in the percentage of body weight increase, final length, and SGR in comparing the group did not receive the prebiotic after one week starvation. Most of fish spends long or short periods of starvation throughout their lives. The effects of starvation periods are different in various species of fish. During the period of feeding limitation, fish consumes the nutrient reserves of the 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 of re-feeding time (Heide, Foss, Stefansson, Mayer, Norberg, Roth, Jenssen, Nortvdt & Imsland 2006). Based on the results of a study which done by Nikbakhsh and Bahrekazemi (2017) at completely similar condition, , the addition of prebiotic MHF-Y at 1, 2, and 3 g in kg of diet of carp led to significant changes in the percentage of body weight increase and final biomass in 2 g of the prebiotic. So, it can be a good supplement for a re-feeding period after starvation too. A research showed that the addition of 1.5 percent inulin to a commercial diet of carp fry could have positive impacts on the growth and survival of carp (Akrami *et al.* 2011). Also, the use of Fermacto prebiotic showed a significant increase in body weight as well as the best SGR, FCR, and PER in carp fed with 3 g of dietary prebiotic compared to the control (Mazurkiewicz, Przybyl & Golski 2008) which corresponds our results. In an experiment on the farmed juvenile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), the researchers reported that by increasing MOS, daily food intake increased and that a level of 4.0 percent MOS led to greater weight gain (Sado, Bicudo & Cyrino 2008).

Abolfathi, Hajiimoradloo, Ghorbani and Zamani (2012) examined the effects of starvation and re-feeding on roach (*Rutilis rutilus caspicus* Yakovlev, 1870*)* and detected

[DOR: 20.1001.1.2345315.2018.4.1.2.6]
[DOI: 10.29252/ijaah.4.1.82]

that fish growth after the starvation period was compensable. Further study on the roach by Taheri and Aliasghari (2012) revealed that the control group showed the highest values in weight gain, daily growth rate, and SGR which corresponds to what found here, but in Black carp (*Mylopharyngodon piceus* J.Richardson, 1846) the study showed that after 21 days of refeeding body mass was greater than of prestarvation but SGR was higher than of control group (Pang *et al*. 2016). In a study on Atlantic cod (*Gadus morhua* Linnaeus, 1758*)*, 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, Meloy, Santos & Christiansen 1994). Similar results were recorded in the pond fish (*Carassius auratus gibelio* Bloch, 1782) (Xie, Zhu, Cui, Wootton, Lei & Yang 2001) and Tinfoil barb (*Barbonymus schwanenfeldii* Bleeker, 1853) after one week starvation (Eslamloo, Azodi & Morshedi 2012).

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, Krumschnalbel & Ojwang-Okwor 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, Nicieza & Wootton 2003). Hence, by increasing the re-feeding duration, the 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. For example, by increasing the starvation period, the amounts of growth and survival rates decreased in Common carp (Assareh *et al.* 2012). 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 Aliasghari (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. In the study done by Nikbakhsh and Bahrekazemi (2017) the FCR values in treatments containing prebiotic Mito decreased, especially at a level of 2 g per kg diet. It is likely that this prebiotic produces

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digestive enzymes (amylase, protease and lipase) as a result of the proliferation of probiotic bacteria and ultimately, reduces the amount of FCR in the host (Tovar, Zambonino & Cahu, Gatesoupe, Vázquez-Juárez, & Lésel 2002).These enzymes eventually render increased digestion of fats and proteins in the diet followed by marked rise in the nutrition efficiency and subsequent growth performance in the host (De Schrijver & Ollevier 2000). Although the amount of FCR was lower than the control group which can be because of one week starvation. Unlike our results, the amount of FCR was not different significantly between treatments in *Barbonymus schwanenfeldii* after one and two weeks starvation (Eslamloo *et al*. 2012).

One of the most reliable indicators of health status and fish physiology is a measurement of blood parameters that is affected by nutrition, environmental factors, age, sex and other physiological parameters (Razeghi Mansour, Akrami, Ghobadi, Amani Denji, Ezatrahimi & Gharaei 2012). In this study, changes in RBC did not show significant differences between the three groups, while the amounts of hemoglobin and hematocrit were higher in the control group. Based on the Kondera *et al*. (2017) results, starvation reduced hematopoietic activity in Common carp which was directly related to the metabolic rate. They reported that the values of most blood parameters did not significantly differ after re-feeding but, RBC frequency was reduced in starved fish. Also, unlike our results, in Indian Major carp refeeding with starvation after 3- 7 days can be use in Rohu culture but these fish had still lower values of hemoglobin, hematocrite and RBC count in comparison to control group (Prabhakar *et al.* 2008). Increased hemoglobin concentration affects the transmissibility of respiratory gases in the blood, the efficiency of the heart, and fish weight gain (Razeghi Mansour *et al*. 2012). Welker, Lim, Yildirim-Aksoy, Shelby and Klesius (2007) examined the effect of probiotic MOS on the catfish (*Ictahurus punctatus* Rafinesque, 1818) 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, Jalali, Sudagar, Azari Takami & Mohammadi Zarajabad 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 (*Oreochromis 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

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parameters compared with the control, which do not agree with our finding. Andrews, Sahu, Pal and Kumar (2009) through the addition of probiotic MOS (1, 2 and 4 percent) in the diet of *Labeo rohita* (Hamilton 1822), fingerlings noticed significant rises in the WBC, RBC, hemoglobin, serum protein, albumin, and globulin in the treated fish compared to the control. In this study, basophils, eosinophils, and monocytes did not show significant differences in the three treatments relative to each other. Del Rio-Zaragoza, Fajer-Avila and Almazan-Rueda (2011) used beta-glucan (0.5, 0.1, and 0.5 percent) in the diet of red snapper (*Lutjanus guttatus* Bloch,1790) for 5 weeks and reported that levels of 0.5 and 0.1 significantly increased monocytes and neutrophils in the 2^{nd} and 4^{th} weeks in comparison to other treatments, which is contrary to the results of this study.

Conclusion

According to the results of this study, although the use of 2 g prebiotic Mito in the kg of diet largely caused compensatory growth in common carp, 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 addition of 0.2% prebiotic Mito in the diet, significantly elevates hematological status of the fish compared to the control group only, which can be used as a recommended suitable complement to the diet.

Conflict of interests

The authors declare that there is no conflict of interest.

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[DOR: 20.1001.1.2345315.2018.4.1.2.6]

[DOI: 10.29252/ijaah.4.1.82]

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[DOR: 20.1001.1.2345315.2018.4.1.2.6]

[DOI: 10.29252/ijaah.4.1.82]

تأثیر تجویز خوراکی پربیوتیک میتو و گرسنگی بر رشد جبرانی، بازماندگی و پارامترهای خونی در ماهی کپور معمولی (*Cyprinus carpio* Linnaeus, 1758)

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چکیده

به منظور بررسی تأثیر پربیوتیک میتو بر رشد جبرانی پس از یک هفته گرسنگی در کپور معمولی، ماهیان (۰/۰۵ ± ۴/۵ گرم) به مدت ۶۰ روز در قالب سه تیمار مورد بررسی قرار گرفتند. گروه کنترل بدون تحمل دوره گرسنگی که غذای فاقد پربیوتیک را دریافت کرد (تیمار اول)، تیمار دوم که بعد از تحمل یک هفته گرسنگی غذای حاوی ۰/۲ درصد پربیوتیک میتو را دریافت کرد و تیمار سوم که بعد از یک هفته گرسنگی غذای فاقد پربیوتیک را دریافت کرد. بیشترین درصد افزایش وزن و ضریب رشد ویژه و ضریب چاقی در تیمار شاهد مشاهده شد که با گروه دوم که در رتبه بعدی قرار داشت دارای تفاوت معنی دار بود ($P <$۰/۰۵) . کمترین مقدار ضریب تبدیل غذایی و بیشترین نسبت کارایی پروتئین نیز در تیمار اول ثبت شد. میزان تلفات در تمام تیمارها صفر درصد بود. در تعداد گلبول‌های قرمز تفاوت معنی‌دار بین تیمارها و گروه کنترل مشاهده نشد ($P >$۰/۰۵). میزان هموگلوبین و هماتوکریت بین گروه شاهد و دو تیمار دیگر به طور معنی‌داری متفاوت بود. بیشترین تعداد گلبول‌های سفید و درصد لنفوسیت در تیمار دوم مشاهده شد در حالیکه در درصد بازوفیل، ائوزینوفیل و مونوسیت تفاوت معنی‌دار بین تیمارها مشاهده نشد ($P >$۰/۰۵). بنابراین اگرچه استفاده از ۰/۲ درصد پربیوتیک توانست تا حد زیادی موجب رشد جبرانی در کپور شود اما نتوانست رشد را به میزان گروه شاهد افزایش دهد. به هر حال استفاده از پربیوتیک میتو توانست شاخص‌های خونی در ماهی کپور را به طور چشمگیری بهبود دهد.

کلمات کلیدی: کپور معمولی، پربیوتیک میتو، شاخص‌های خون شناسی، رشد جبرانی، بازماندگی

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[DOR: 20.1001.1.2345315.2018.4.1.2.6]

[DOI: 10.29252/ijaah.4.1.82]