

Effect of oral administration of Levamisole, Quil-A and Cinnamon in growth amount, hematological and immune parameters of Marmalade cichlid, *Labeotrophus fuelleborni* (Ahl, 1926)

Z Amiri¹, M Bahrekazemi ^{1*}

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

Received: August 2017

Accept: October 2017

Abstract

The study evaluated the effects of Levamisole, Quil-A and Cinnamon on growth, hematological and immune parameters of marmalade cichlid. Fish (2.00 ± 0.02 g) were fed diets supplemented with 0.2% Levamisole, 0.5% Quil-A and 1% Cinnamon for 60 days and the control group was fed with basal diet. The highest weight gain and condition factor were observed in fish fed diet enriched with Levamisole followed by Quil-A. There was no significant difference between the three treatments in specific growth rate (SGR) ($p>0.05$). However, the SGR showed a significant difference in the control group ($2.16 \pm 0.02\% \text{ day}^{-1}$) compared to other treatments ($p<0.05$). No significant difference in survival rate was observed between treatments ($p>0.05$).

***Correspondence** M Bahrekazemi, Department of Fisheries, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran. (e-mail: bahr.kazemi@gmail.com).

The lowest FCR was recorded in fish fed diet enriched with Levamisole (1.74 ± 0.95). The highest levels of RBCs, hemoglobin and hematocrit were observed in Levamisole treatment, but the highest number of WBCs was recorded in Cinnamon treatment ($16766700 \pm 450.92 \text{ mL}^{-1}$). The maximum level of lysozyme was also observed in Cinnamon treatment ($8.84 \pm 0.38 \mu\text{g mL}^{-1}$). While no significant difference was recorded in the levels of the basophil, eosinophil, and monocyte, the highest levels of neutrophils ($25.00 \pm 1.0\%$) and lymphocytes ($78.33 \pm 0.57\%$) were observed in Cinnamon treatment. So, the use of Levamisole significantly improved growth performance and hematological parameters, but immunological parameters were improved with fish fed diet supplemented with Cinnamon.

Keywords: Marmalade cichlid, Cinnamon, Quil-A, Levamisole, Hematology, Immune factors

Introduction

Reducing the survival rate of fishes, is one of the most important issues that fish breeders are faced with, especially in the early stages of fish life. This is more important for ornamental fish that are kept in the aquarium and usually with high density (Srinivasan 2013). Therefore, induction of the fish immune system, especially in valuable and economical species, is one of the most basic needs for fish breeders and is the most important approach of researchers (Roberts 2009). In addition, the aquaculture industry has been affected by the incidence of epidemic diseases along with the development and progression of this industry, so that it has become difficult to control such diseases today (Shalaby, Khattab & Abdel Rahman 2006).

Many years ago, many medicines have been prescribed to treat various contaminations of fish, especially bacterial one. However, overcoming the bacterial resistance to antibiotics as well as pollution effects on the environment is one of the most important problems with the use of these medicines in aquaculture. The production of the vaccine against many of the intracellular pathogens and many viruses has not been yet successful. Therefore, control of all diseases in fish is not possible only using vaccination (Tafi & Meshkini 2014). This problem led researchers to use of many chemicals and natural compounds as a stimulant and immune booster in aquatics (Alishahi, Mesbah, Namjouyan, Sabzevarizadeh & Razi Jalali 2012). Immuno stimulants facilitate the action of phagocytosis

and increase the antibacterial activity of fish. Also, the stimulation of phagocytic cells, complement proteins, lysozyme and antibody responses can actually increase the resistance of fish to various diseases and protects aquatic organisms against bacterial agents (Tafi & Meshkini 2014). Immuno stimulants also can enhance resistance to stressors such as oxygen deficiency, temperature, salinity as well as improvement of feed conversion ratio (FCR) and other growth parameters. In fact, these compounds are able to compensate deficiencies and weaknesses of fish caused by stressors (Kim & Rajapakse 2005).

Up to now, oral administration has been reported to prescribe immune stimulants such as Glucan, Lactoferrin, Levamisole, Chitosan and plant extracts. Oral administration of immune stimulants would have many advantages to fish such as being stress-free and use of stimulants, regardless of fish size (Raa 1996). It should be noted that some of the plants are a source of tannins, polysaccharides, alkaloids, flavonoids and saponins that may have antimicrobial effects and may even strengthen the immune system of fish. In recent years, willingness to replace chemical stimulants with less harmful and cheaper materials such as thyme, aloevera, black-caraway, etc., has been increased (Ardo, Yin, Xu, Varadi, Szigeti & Jeney 2008; Alishahi *et al.* 2012).

So far, the effects of different immune stimulants have been investigated in ornamental fishes, such as use of Levamisole in Oscar fish

(*Astronotus ocellatus* Agassiz, 1831) (Alishahi *et al.* 2012), Cinnamon powder in Green terror (*Andinocara rivulatus* Gunther, 1860) (Roozi, Mooraki, Zorih Zahra & Haghghi 2013) and Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) (Ahmad, El Mesallamy, Samir & Zahran 2011), Quil-A in Tilapia (*O. niloticus*) (Francis, Makkar & Becker 2001) and Japanese flounder (*Paralichthys olivaceus* Temminck & Schlegel, 1846) (Chen, Ai, Mai, Xu, Liufu, Zhang & Cai 2011), *Zhumeria majdae* (Rech & Wendelbo, 1967) and *Salvia officinalis* (Linnaeus, 1753) in Catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) (Rezaii, Sourinejad, Soltanian & Yousefzadi 2013). Thus, the aim of this study was to evaluate the effects of one chemical [Levamisole] and two natural immune stimulants [Quil-A (*Quillaja saponaria* Molina, 1911) and Cinnamon (*Cinnamomum zeylanicum* Blume, 1897)] on growth performance, hematological and immune parameters of Marmalade cichlid (*Labeotropheus fuelleborni*), which is one of the most important

and most valuable freshwater ornamental fish, and compare their effects.

Materials and Methods

In the Ornamental Fish Research Center (Jooybar-Mazandaran) in winter of 2016, Three hundred juvenile marmalade cichlid with an average weight of 2 g, transferred to the laboratory, then acclimatized in aquariums (0.6 m³) for two weeks. The water was provided from well (water quality conditions are given in Table 1). After this period, fish juvenile were randomly distributed into 12 aquariums (with a volume of 100 liters and the density of 15 fish in each aquarium). This study included a control group that did not receive any immune stimulant in corporate with the basal diet, and three other treatments were as follows: a diet containing 0.2% Levamisole (Alishahi *et al.* 2012), a diet containing 0.5% Quil-A (Francis *et al.* 2001) and a diet containing 1% Cinnamon powder (Ahmad *et al.* 2011).

Table 1. Water quality parameters during the 60 days of experiment

Parameters	Range
Temperature (°C)	23-25
Dissolved oxygen (ppm)	7.6-8.4
water hardness (mg L ⁻¹ CaCO ₃)	350
pH	7.5

The ingredients and chemical composition of the basal diet (Abzian Shomal, Iran), are shown in Table 2. To prepare the experimental diets containing 2 mg kg⁻¹ Levamisole, 5 mg kg⁻¹ Quil-A and 10 mg kg⁻¹ Cinnamon, were separately dissolved in 15 mL of distilled water. Prepared solutions were then sprayed with special sprinklers the basal diet for each

treatment. Then, experimental diets were dried at room temperature and stored at 4 °C until use. The control diet was prepared to add only 15 mL of distilled water to the basal diet. So, the only difference between control diet and three other experimental diets was the presence of immune stimulants (Alishahi *et al.* 2012).

Table 2. Ingredients and proximate analysis of the experimental diet (%) of Marmalade cichlid used in this study

Ingredients	Percent
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	36.23
Crude lipid	11
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.

The fish were fed twice a day with laboratory prepared feed at the rate of 4% of their body weight. At the end of the 60-day period of the experiment, all fish were deprived of food for 24 h before weighing and sampling. Parameters were measured at the end of feeding trial (Boujard, Labbe & Auperin 2002):

$$\text{Weight gain (g)} = \text{Wf (g)} - \text{Wi (g)}$$

$$\text{SGR (\% day}^{-1}) = \frac{(\ln \text{Wf} - \ln \text{Wi}) * 100}{t}$$

$$\text{Condition Factor (CF)(\%)} = (\text{Wf/Lf}^3) * 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

Survival rate (%)

$$= \frac{\text{number of fish at the end of the period}}{\text{number of fish at the beginning of the period}} * 100$$

Where Wf is final weight mean, Wi is initial weight mean, Lf is final length mean and t is the period of study.

At the end of the experiment, five fish were sampled randomly from each aquarium and were anesthetized with 1.0 g per liter clove solution (Nikbakhsh & Bahrekazemi 2017),

and blood was drawn from the caudal vein, using a 2 mL syringe. Then, blood samples were introduced to both heparinized and non-heparinized tubes in order to perform hematological and immunological studies, respectively. The erythrocyte (RBC) and leukocyte (WBC) counts were determined using a Neubauer hemocytometer (Houston, Dobric & Kahurananga 1996). Hematocrit (Hct) was measured using the standard microhematocrit method (Rehulka 2000). Hemoglobin levels (Hb) were obtained using the standard kit and a spectrophotometer (Hettich, Germany) at a wave length of 540 nm (Blaxhall & Daisley 1973). Differential leukocyte counts were determined using Giemsa staining method and detected under a light microscope (Alishahi *et al.* 2012). Serum lysozyme levels were also measured using the Turbidimetric Method (Eliss 1990).

In order to analyze the data, a normalization test (Shapiro-Wilk test) was first performed.

The data were analyzed using one-way ANOVA. The significant differences between treatment were analyzed at 95% confidence level ($p<0.05$) and mean separation was

conducted by Duncan's multiple range test. The data were processed in Microsoft Excel 2010, then all statistical analyzes were done using the SPSS software package version 19.

Results

Growth performance and survival

The highest weight gain was observed in fish fed diet enriched with Levamisole followed by Quil-A which had no significant difference with each other ($p>0.05$), but showed significant difference with two other treatments ($p<0.05$). The minimum weight gain belonged to the control group. There was no significant difference between three experimental treatments (Levamisole, Quil-A and Cinnamon) in case of specific growth rate (SGR). However, the SGR showed a significant difference in the control group compared to other treatments. The highest amount of condition factor (CF) was recorded on treatment fed with Levamisole followed by Quil-A which had no significant difference

with each other ($p>0.05$), while these two treatments had a significant difference with control group and fish fed with Cinnamon ($p<0.05$) (Table 3). The feed conversion ratio (FCR) was improved in those fish fed with the diet containing the immune stimulants over the control. The lowest FCR was recorded in fish fed with Levamisole followed by treatment fed with Quil-A ($p>0.05$) (Table 3).

At the end of the experiment, there was no significant difference in survival rate between treatments compare with the control group ($p>0.05$), however, the highest amount of this index was recorded on fish fed with Levamisole ($94.44 \pm 7.69\%$) (Table 3).

Table 3. Growth performance of Marmalade cichlid fed diets enriched with immune stimulants

Treatments	Body Weight gain (g)	SGR (% day ⁻¹)	CF (%)	FCR	Survival rate (%)
Control	6.04 ± 0.23^c	2.16 ± 0.02^b	1.39 ± 0.005^c	2.52 ± 0.85^a	90.00 ± 0.00^a
Quil-A	8.66 ± 0.24^{ab}	2.60 ± 0.05^a	1.51 ± 0.01^{ab}	1.85 ± 0.51^{bc}	82.21 ± 7.69^a
Levamisole	9.20 ± 0.52^a	2.68 ± 0.12^a	1.52 ± 0.01^a	1.74 ± 0.95^c	94.44 ± 7.69^a
Cinnamon	8.45 ± 0.24^b	2.68 ± 0.02^a	1.49 ± 0.01^b	1.89 ± 0.56^b	90.00 ± 0.00^a

Values in each column with different superscripts show significant difference ($p<0.05$).

Hematological and immunological analysis

The number of total red blood cells was increased in a statistically significant manner on fish fed diets enriched with immune stimulants. The lowest number of RBC was observed in control group ($p<0.05$). The minimum and

maximum number of white blood cells were recorded in control group and fish fed diets enriched with Cinnamon, respectively ($p<0.05$). Hemoglobin and hematocrit values were also increased in all groups of fish fed diets enriched

with immune stimulants, respect to the values found in control group ($p<0.05$). In this regard, fish fed diet enriched with Levamisole contained the highest amounts of hemoglobin (5.61 ± 0.03

g dL^{-1}) and hematocrit. The lowest amounts of hemoglobin ($21.00 \pm 1 \text{ g dL}^{-1}$) and hematocrit have also belonged to the control group (Table 4).

Table 4. Hematological parameters of marmalade cichlid fed diets enriched with immune stimulants for 60 days

Treatments	RBC (10^6 mL^{-1})	WBC (10^3 mL^{-1})	Hct (%)	Hb (g dL^{-1})
Control	1.43 ± 0.03^b	11100.00 ± 360.55^c	21.00 ± 1^c	4.99 ± 0.06^c
Quil-A	1.59 ± 0.08^a	11533.3 ± 416.33^c	23.00 ± 0.17^b	5.16 ± 0.01^b
Levamisole	1.60 ± 0.04^a	13500.00 ± 300^b	25.66 ± 0.57^a	5.61 ± 0.03^a
Cinnamon	1.65 ± 0.02^a	16766.70 ± 450.92^a	25.33 ± 0.57^a	5.17 ± 0.01^b

Values in each column with different superscripts show significant difference ($p<0.05$).

The results showed the highest amount of lysozyme (8.84 ± 0.38) was significantly belonged to the fish fed diet enriched with Cinnamon. The control group showed the lowest amount of lysozyme. The results of differential WBC counts indicated that there was no

significant difference in basophil, eosinophil and monocyte levels between four groups ($p>0.05$), but the highest levels of neutrophil ($25.00 \pm 1\%$) and lymphocyte ($78.33 \pm 0.57\%$) were recorded on fish fed diet enriched with Cinnamon ($p<0.05$) (Table 5).

Table 5. Immunological parameters of marmalade cichlid fed diets enriched with immune stimulants for 60 days

Treatments	Lysozyme ($\mu\text{g mL}^{-1}$)	Basophil (%)	Eosinophil (%)	Neutrophil (%)	Monocyte (%)	Lymphocyte (%)
Control	7.21 ± 0.27^b	1.33 ± 0.57^a	4.66 ± 0.57^a	15.33 ± 0.57^c	0.66 ± 0.57^a	68.33 ± 0.57^a
Quil-A	7.45 ± 0.15^b	1.66 ± 0.57^a	4.33 ± 0.57^a	24.66 ± 1.15^a	1.00 ± 0.57^a	70.33 ± 1.52^c
Levamisole	7.66 ± 0.15^b	1.33 ± 0.57^a	4.0 ± 0.57^a	20.33 ± 0.57^b	0.66 ± 0.57^a	73.33 ± 0.57^b
Cinnamon	8.84 ± 0.38^a	1.33 ± 0.57^a	4.00 ± 0.57^a	25.00 ± 1.0^a	0.66 ± 0.57^a	78.33 ± 0.57^a

Values in each column with different superscripts show significant difference ($p<0.05$).

Discussion

In this study, the maximum increase in body weight, specific growth rate and condition factor, were recorded on a fish fed diet enriched with Levamisole, although there was no significant difference between Levamisole and Quil-A. However, there was a significant difference between two mentioned groups and two other groups. In line with the present study, Alishahi *et al.* (2012) reported that use of

Levamisole can increase the growth rate of Oscar fish (*Astronotus ocellatus*). Also, the use of Levamisole in *Clarias fuscus* (Lacepede, 1803) can cause increased weight gain (Guifeng, Yungui, Dianhui, Peifeng, Jijia, Cuie, Lanqing & Haifang 2006). These findings were similar to the results of this study. In a study conducted on Tilapia (*O. niloticus*) and Common carp (*Cyprinus carpio* Linnaeus, 1758),

the researchers concluded that the using of Quil-A can increase body weight of this fish by up to 20 percent. They showed that this fish can gradually get used to more than 700 mg kg⁻¹ of Quil-A in their diet (Francis, Makkar & Becker 2002c). The presence of this type of saponin (Quil-A) can further increase the penetration of enzymes into the diet, by modulating the viscosity of digestive juice, which ultimately improves the growth performance. In fact, using natural immune stimulants such as Quil-A can increase the intake of food and growth rate by increasing the metabolic rate (Rezaii *et al.* 2013). Although, Cinnamon powder increased growth rate compared to the control group, but it was significantly less than two other treatments. The results of a study used 1% Cinnamon in the diet of Nile tilapia, showed that there was an increment in specific growth rate compared to the control group (Ahmad *et al.* 2011). In the present study, condition factor was significantly increased in experimental treatments compared to control group. The highest amount of condition factors were observed on fish fed diets enriched with Levamisole and Quil-A. In fact, the growth performance was affected by body weight gain, which is also dependent on palatability of food and increase of food intake.

One of the factors which makes aquaculture to be economic is lowering the FCR, which results not only in economical consumption of food and feed costs but also in preventing the secondary pollution of the environment and consequently, reduced the water quality parameters (Falahatkar, Soltani, Abtahi,

Kalbasi, Poor Kazemi & Yasemi 2006). In this study, the fish that received immune stimulants in their diets showed lower levels of FCR compared to the control group, which means better feed efficiency and nutrient digestibility of diet. In fact, immune stimulants stimulate active transport of oxygen molecules across the cell membrane. As a result, metabolism of cells was done at a higher level, therefore consumption of food and FCR can be improved (Alishahi *et al.* 2012). Francis *et al.* (2001 & 2002c) reported that using of Quil-A can reduce the FCR in Nile tilapia and Common carp. Increasing up to 6.6 mg kg⁻¹ Quil-A in the diet of Japanese flounder (*Paralichthys olivaceus*) led to increased body weight and reduced FCR (Chen *et al.* 2011). Similar results were obtained using Cinnamon in the diet of Nile tilapia (Ahmad *et al.* 2011). These finding were similar to ours.

The results showed that there was no significant difference in survival rate between treatments, which is similar to the results of Alishahi *et al.* (2012) on Oscar fish. The survival rate indicates immunity against pathogens and environmental stressors, but it should be noted that using immune stimulants usually will cause significant changes the survival rate of fish over a period of more than six months (Borges, Scotti, Siqueira, Jurinitz & Wassermann 2004).

Hematological parameters are used as an index of fish health status in fish species to detect potential effects of some anti-nutritional agents. Changes in hematological parameters are one of the reactions that animals show in

response to stress. A part of these changes depends on characteristics of the red blood cells, such as changes in cell size and hemoglobin levels, and the other part depends on the plasma concentration, which can change the number of blood cells per unit volume, as well as change in hematocrit levels (Lin, Pan & Luo 2011).

In this study, use of all three immune stimulants (Levamisole, Quil-A and Cinnamon) was able to significantly increase the number of RBCs in the blood of Marmalade cichlid. In line with this study, Roozi *et al.* (2013) reported that use of 1% Cinnamon powder in the diet of Green terror can increase the number of RBCs. Ahmed *et al.* (2011) reported that the use of Cinnamon in the diet of Nile tilapia also significantly increased the number of RBCs. Also, Maqsood, Samoon & Singh (2009) showed that oral use of Levamisole in Common carp caused an increase in RBCs, hematocrit, and hemoglobin. But in a study on the use of Levamisole in Oscar fish, the results showed no significant difference in these parameters (Alishahi *et al.* 2012).

The WBCs, especially the B and T lymphocytes, play an important role in the defense system of fish. A change in the number of these cells seems to be reasonable by using immune stimulants. An increase in the number of WBCs has been reported under natural and experimental infections and in the use of various vaccines and immune stimulants (Marian 2004). In this study, the maximum number of WBCs was observed in Cinnamon treatment followed by Levamisole treatment.

Roozi *et al.* (2013) reported that use of Cinnamon powder in the diet of Green terror (*Andinocara rivulatus*) significantly increased the number of WBCs, which finally improved the immune system of fish. According to their findings, it seems that effect of Cinnamon on stimulating and strengthening of the immune system is due to antimicrobial effect against pathogenic agents and an increased immune response by affecting the number of WBCs. Alishahi *et al.* (2012), reported that the use of Levamisole as a nutritional supplement in the diet of Oscar fish enhanced the number of WBCs, which is similar to the result of our Levamisole treatment.

In this study, the highest levels of hematocrit and hemoglobin were measured in Levamisole treatment. Hemoglobin and hematocrit levels change with RBCs changes and have a direct relationship with it. Increased hemoglobin concentration in fish has an effect in controlling the transmission of respiratory gases in the blood, cardiac efficiency and weight gain (Gazerani Farahani 2009). Roozi *et al.* (2013), reported that the use of Cinnamon in the feeding of green terror can significantly increase the level of hemoglobin. Also, the use of Cinnamon in the diet of Nile tilapia significantly increased the hemoglobin level (Ahmad *et al.* 2011), which is similar to our findings. In Marmalade cichlid, the addition of 1% Cinnamon, increased hemoglobin and hematocrit levels compared with the control group.

Our results showed that lysozyme significantly increased under the influence of

Cinnamon using the diet. As a non-specific immunity marker in fish (Sakai 2000), the amount of lysozyme, significantly increased in fish fed diet enriched with Cinnamon compared to three other treatments. Several factors affect the antibacterial activity of lysozyme, including sexual maturity, season, age, environmental temperature and the use of stimulants (Soltani & Pourgholam 2007). An increase in lysozyme levels has been reported with the use of Levamisole in *Clarias fuscus* (Guifeng *et al.* 2006). Levamisole also increased the amount of lysozyme in Indian carp (*Labeo rohita* Hamilton, 1822) (Wijendra & Pathiratne 2007). It should be noted that we did not find any report about the effect of Cinnamon on lysozyme level, so we could not compare our results. In this study, no significant difference was observed in the basophil, eosinophil, and monocyte. Also, the use of Cinnamon powder in the diet of green terror had no effect on the number of basophils and eosinophils (Roozi *et al.* 2013). Neutrophils are a group of blood cells that have phagocytic properties, so these cells increased immunity against to bacterial diseases. In this study, the fish that received Cinnamon in their diet showed the highest levels of lymphocyte and neutrophil. This stimulant, increases the production of interferons, interleukins and complement proteins which leads to increase the lymphocyte level (Sakai 2000). Similar to our results in Marmalade cichlid, Akhlaghi and Anbaraki Motlagh (2004) reported that the use of Quil-A can increase the lymphocyte level in Common carp.

Conclusion

In terms of growth parameters, Levamisole and Quil-A had the maximum effect, although Levamisole significantly improved hematological parameters. Also, immunological parameters were improved in fish fed diet enriched with Cinnamon compared to other treatments.

References

Ahmad M. H., El Mesallamy A. M. D., Samir F. & Zahran, F. (2011) Effect of Cinnamon (*Cinnamomus zeylanicum*) on growth performance, feed utilization, whole body composition, and resistance to *Aeromonas hydrophila* in Nile tilapia. *Journal of Applied Aquaculture* 23, 289–298.

Akhlaghi M. & Anbaraki Motlagh M. (2004) Phagocytosis changes in Common carp, following the use of Quil-A and Levamisole. *Iranian Scientific Fisheries Journal* 13 (3), 1-12.

Alishahi M., Mesbah M., Namjouyan F., Sabzevarizadeh M. & Razi Jalali M. (2012) Comparison the effects of some chemical and natural immune stimulants on Oscar fish (*Astronotus ocellatus*). *Iranian Veterinary Journal* 8 (2), 58-69. [In Persian].

Ardo L., Yin G., Xu P., Varadi L., Szigeti G. & Jeney Z. (2008) Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture* 275, 26-33.

Blaxhall P. C. & Daisley K. W. (1973) Routine hematological methods for use with fish bloods. *Journal of Fish Biology* 5, 771-781.

Borges A., Scotti L. V., Siqueira D. R., Jurinitz D. F. & Wassermann G. F. (2004) Hematological and serum biochemical values for jundia (*Rhamdia quelen*). *Fish Physiology and Biochemistry* 30, 21-25.

Boujard T., Labb   L. & Aup  rin B. (2002) Feeding behaviour, energy expenditure and growth of rainbow trout in relation to stocking density and food accessibility. *Aquaculture Research* 33, 1233-1242.

Chen W., Ai O., Mai K., Xu W., Liufu Z., Zhang W. & Cai, Y. (2011) Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 318, 95-100.

Eliss A.E. (1990) Lysozyme assay. In: Stolen J.S., Fletcher D.P., Anderson B.S. & Robertson B.S. (eds). *Techniques in Fish Immunology*, pp.101-103. Fair Haven, N J, USA SOS Publication.

Falahatkar B., Soltani M., Abtahi B., Kalbasi M.R., Poor Kazemi M. & Yasemi M. (2006) Effects of vitamin C on some growth parameters, survival and hepatosomatic index in juvenile cultured beluga, *Huso huso*. *Veterinary Journal* 72, 98-103. [In Persian].

Francis G., Makkar H.P.S. & Becker K. (2002c) Effects of cyclic and regular feeding of Quillaja saponin supplemented diet on growth and metabolism of Common carp (*Cyprinus carpio*). *Fish physiology and Biochemistry* 24, 343-350.

Francis G., Makkar H.P.S. & Becker K. (2001) Effects of Quillaja saponin on growth, metabolism, egg production, and muscle cholesterol in individually reared Nile tilapia (*Oreochromis niloticus*). *Comparative Biochemistry and Physiology* 129, 105-114.

Gazerani Farahani M. (2009) Evaluation of some hematological parameters in some species of Acipenseridae. *Journal of Animal Biology* 2 (1), 12-23. [In Persian].

Guifeng L., Yungui G., Dianhui Z., Peifeng Q., Jijia S., Cuie X., Lanqing L. & Haifang W. (2006) Effects of Levamisole on the immune response and disease resistance of *Clarias fuscus*. *Aquaculture* 253 (1-4), 212-217.

Houston A.H, Dobric N. & Kahurananga R. (1996) The nature of hematological response in fish. Studies on rainbow trout *Oncorhynchus mykiss* exposed to stimulated winter, spring and summer conditions. *Fish Physiology and Biochemistry* 15, 339-347.

Kim S. K. & Rajapakse N. (2005) Enzymatic production and biological activities of chitosan oligosaccharides (COS): a review. *Carbohydrate Research* 62, 357-368.

Lin S., Pan Y., Luo L. & Luo L. (2011) Effects of dietary B-1,3-glucan, chitosan or raffinose on the growth, innate immunity and resistance of koi (*Cyprinus carpio koi*). *Fish and Shellfish Immunology* 31, 788-794.

Marian M.P. (2004). Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. *Aquaculture* 237, 9-20.

Maqsood S., Samoon M.H. & Singh P. (2009). Immunomodulatory and growth promoting effect of dietary Levamisole in *Cyprinus carpio* fingerlings against the challenge of *Aeromonas hydrophila*. *Turkish Journal of Fisheries and Aquatic Sciences* 9, 111-120.

Nikbakhsh J. & Bahrekazemi M. (2017) Effect of diets containing different levels of prebiotic Mito on the growth factors, survival, body composition, and hematological parameters in common carp *Cyprinus carpio* fry. *Journal of Marine Biology and Aquaculture* 3 (1), 1-6.

Raa J. (1996). The use of immune stimulatory substance in fish and shellfish farming. *Fisheries Science* 4 (3), 229-288.

Rehulka J. (2000) Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 190, 27-47.

Rezaii M.H., Sourinejad I., Soltanian S. & Yousefzadi M. (2013) The effects of dietary *Zhumeria majdae* extract on growth indices, hematology and immunology of Catfish *Pangasianodon hypophthalmus*. *Journal of Aquatic Ecology* 3 (1), 8-19. [In Persian].

Roberts H.E. (2009) Fundamentals of ornamental fish health. Wiley- Blackwell, 244.

Roozi Y., Moraky N., Zorih Zahra S.G. & Haghghi M. (2013) Study the effects of different levels of Cinnamon powder (*Cinnamomum zeylanicum*) on hematological parameters, blood glucose and survival rate of Green terror (*Andinocara rivulatus*). *Quarterly Journal of Applied Sciences and Aquaculture* 1 (3), 41-52.

Sakai M. (2000) Current research status of fish immuno stimulants. *Aquaculture* 172, 63-92.

Shalaby A. M, Khattab Y.A. & Abdel Rahman A.M. (2006) Effect of garlic (*Allium sativum*) and Chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). *Journal of Venomous Animals and Toxins* 12, 172-201.

Soltani M. & Pourgholam R. (2007) Lysozyme activity of Grass carp (*Ctenopharingodon idella*) following exposure to sub lethal concentrations of organophosphate, diazinon. *Journal of Veterinary Research* 62 (2), 49-52.

Srinivasan M. (2013) A complete manual on ornamental fish culture. Lambert Academic Publishing, 310.

Tafi A.A. & Meshkini S. (2014) Immune stimulants and their importance in aquaculture. *Journal of Agricultural Engineering and Natural Resources* 46, 48-53. [In Persian].

Wijendra G.D. & Pathiratne A. (2007) Evaluation of immune responses in an Indian carp, *Labeo rohita* fed with Levamisole incorporated diet. *Journal of Science of the University of Kelaniya* 3, 17-28.

تأثیر تجویز خوراکی لوامیزول، کوئیل آ و دارچین بر مقدار رشد، پارامترهای خونی و

ایمنی در سیچلید مارمالاد (*Labeotrophus fuelleborni*)

زینب امیری^۱، معصومه بحر کاظمی^{*}^۱

^۱ گروه شیلات، واحد قائم‌شهر، دانشگاه آزاد اسلامی، قائم‌شهر، ایران

چکیده

این تحقیق اثر لوامیزول، کوئیل آ و دارچین بر فاکتورهای رشد، خون شناسی و ایمنی در ماهی سیچلید مارمالاد را بررسی کرد. ماهیان (0.02 ± 2 گرم) به مدت ۶۰ روز با غذای شاهد (بدون محرک ایمنی) و نیمارهای غذایی حاوی $0.2/0.05$ درصد لوامیزول، $0.5/0.05$ درصد کوئیل آ و $1/0.05$ درصد پودر دارچین تغذیه شدند. بیشترین افزایش وزن و ضریب چاقی در تیمار لوامیزول بدست آمد که با تیمار کوئیل آ تفاوت معنی‌داری نداشت ($p > 0.05$). نرخ رشد ویژه در سه تیمار دریافت‌کننده محرک ایمنی با یکدیگر تفاوت معنی‌داری نداشتند ($p > 0.05$) اما با گروه شاهد ($0.02/0.05$ درصد در روز) دارای تفاوت معنی‌دار بودند ($p < 0.05$). در شاخص زنده‌مانی اختلاف معنی‌داری در بین تیمارها مشاهده نشد ($p > 0.05$). کمترین ضریب تبدیل غذایی در تیمار لوامیزول حاصل شد (0.95 ± 0.74). بیشترین میزان گلbul‌های قرمز و هموگلوبین و هماتوکریت در تیمار لوامیزول اما بیشترین تعداد گلbul‌های سفید ($450/92 \pm 16766700$ در میلی‌لیتر) در تیمار دارچین اندازه‌گیری شد. بالاترین میزان لیزوژیم نیز مربوط به تیمار دارچین بود (0.38 ± 0.04 میکروگرم در میلی‌لیتر). همچنین با وجود معنی‌دار نبودن درصد بازوفیل، اوزینوفیل و مونوسیت بین تیمارها، بیشترین درصد نوتروفیل ($0.00/0.00 \pm 25/0.00$ درصد) و لنفوسیت (0.057 ± 0.033 درصد) در تیمار دارچین مشاهده شد. بنابراین از نظر فاکتورهای رشد و خونی تیمار لوامیزول نتایج بهتری حاصل کرد، اما نقش ایمنی‌زایی دارچین بیشتر از دو محرک دیگر بود.

کلمات کلیدی: سیچلید مارمالاد، دارچین، کوئیل آ، لوامیزول، خون شناسی، فاکتورهای ایمنی

***نویسنده مسئول:** bahr.kazemi@gmail.com