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Effect of Zataria multiflora Boiss on haematological and Growth parameters of Oncorhynchus mykiss

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Abstract

Zataria multiflora is well known to middleeast, Iran, Afghanistan and Pakistan where, it is cultivated only in warm areas. The objective of this article focused on this issue whether Zataria multiflora Boiss has effect on haematological and Growth parameters of Oncorhynchus mykiss. One hundred and twenty healthy finger ling fish, Oncorhynchus mykiss (mean weight 4.3 g) were commercially obtained and then transferred to the research field located in Tehran. Fish were fed on diets covering 0, 50, 100 and 150 mg kg⁻¹ Z. multiflora (Z.M.) as four groups; in triplicates. All of them were hand-fed (3-4% of body weight) to satiation 6-8 times a day for 8 weeks (60 days). There was no significant difference (p>0.05) among the fish fed the different levels of Z.M for the mean weight. The maximum value of SGR was observed in group 50 ppm of Z.M. (2.85 ± 0.01) with significant difference (p < 0.05) with control (2.44 ± 0.0) .

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Accordingly, this result showed that FCR value was minimum in control (7.20 ± 0.0) with no significant difference (p> 0.05) compared with group 50 ppm of Z.M. The result of WG for group 50 ppm of Z.M. (453.86 ± 0.46) showed a significant difference (p< 0.05) with those of other groups. On the other hand, Z.M. in concentration of 50 ppm could enhance WBC compared to control and other treatments as well as RBC but no difference was observed among the treatments (p > 0.05). Against, the values for Hb and MCH were significantly lower than others (p< 0.05). It is concluded that the fish fed with 50 ppm of Zataria multiflora increased mean weight of rainbow trout while enhancement of innate immunity significantly was occurred.

Keywords: *Oncorhynchus mykiss*, *Zataria multiflora*, Weight, Haematological indices.

Introduction

Nowadays, application the herbs seems to be the most auspicious strategy in aquaculture for preventing diseases and weight increase of

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fishes (Ardó, Yin, Xu, Váradi, Szigeti, Jeney & Jeney 2008). Among these herbs, Zataria multiflora is well known to middle-east, Iran, Afghanistan and Pakistan where, it is cultivated only in warm areas (Fazeli, Amin, Attari, Ashtiani, Jamalifar & Samadi 2007, Saei-Dehkordi, Tajik, Moradi & Khalighi-Sigaroodi 2010). Its Persian name, Avishane Shirazi has been traditionally used and prescribed for flavoring and preserving foods (Fazeli et al. 2007), antibacterial and antioxidant activities (Sharififar, Moshafi, Mansouri, Khodashenas & Khoshnoodi 2007), inflammatory bowel disease (Nakhai, Mohammadirad, Minaie, Nikfar, Ghazanfari, Zamani, Dehghan, Jamshidi & Boushehri 2007), anti-candidiasis (Mahmoudabadi, Dabbagh & Fouladi 2007), denture stomatitis treatment (Mahmoudabadi et al. 2007), antitumor activity (Ali, Saleem, Ali & Ahmad 2000) and antispasmodic effect (Gharib Naseri. Mazlomi. Goshaiesh. Vakilzadeh & Heidari 2010). Zataria multiflora, a herb belonging to the Laminaceae family has shown nutritional and medicinal properties (Misaghi & Basti 2007). Carvacrol and Thymol, which are belonging to phenolic and has constituents antibacterial antifungal activities, are the most important components of Z. multiflora essential oil (Ali et al. 2000, Misaghi & Basti 2007). Results of some investigations as follows showed Increase of survival rate and hatchability of rainbow trout eyed-eggs, immunity enhancement and preventing the bacterial growth such as Staphylococcus aureus in the fillet of salinated fish (Choobkar, Soltani, Ebrahimzadeh Mousavi, Akhonzadeh Basti & Matinfar 2010),

reduction or prevention the fungal infection in rainbow trout eggs (Sharif Roohani 2007), some blood and serum parameters in Acipenser persicus (Sharif Rohani, Masoumzadeh, Haghighi, Jalilpoor, Pourdehghani, Shenavar Masouleh, Alizadeh & Bazari Moghaddam 2013). These properties could be due to Luteolin (Ali et al. 2000), water-soluble quercetin (Shafiee, Javidnia & Tabatabai 1999) Terpeneids consists of a mixture of terpenes, oxygenated terpenes, sesquiterpenes and oxygenated sesquiterpenes (Ali et al. 2000, Ebrahimzadeh, Yamini, Sefidkon, Chaloosi & Pourmortazavi 2003, Misaghi & Basti 2007).

The objective of this article focused on this issue whether *Zataria multiflora* Boiss has effect on haematological and Growth parameters of *Oncorhynchus mykiss*.

Materials and Methods

Fish

One hundred and twenty healthy finger ling fish, *Oncorhynchus mykiss* (mean weight 4.3 g) were commercially obtained from a private sector in Tehran, Iran. They were then transferred to the research field located in Biotechnology Institute in Tehran, where was designed by our research team. The fish addaptated in a holding veniro prefilled with a well water for two weeks before experiment.

They were randomly distributed among twelve 2000-L high density polyethylene (HDPE) tanks at a density of 10 fish each. The tanks were prefilled with 400 L of clean well-aerated well-water (120 L h⁻¹ of flow rate). The water was maintained at 15.5 °C, dissolved

oxygen 7.5 mg L⁻¹, pH 8.1. Fish were fed on diets covering 0, 50, 100 and 150 mg kg⁻¹ *Z. multiflora* (Z.M.) as four groups; in triplicates. All of them were hand-fed (3-4% of body weight) to satiation 6-8 times a day for 8 weeks (60 days).

Experimental diet and herbal extract

fish food was prepared based on the method of Kakoolaki, Akbary, Zorriehzahra, Salehi, Sepahdari, Afsharnasab, Mehrabi and Jadgal (2016) with the basis of the Kimiagaran-e-Taghzieh company (protein 42-46%, fat 15%, fiber 1.5-2.5%, 10% ash, 10% moisture content). The Z.M. powder was then added to the basal control diet with spraying of olive oil, to obtain 4 different treatments as 0.0 (Control group) 50, 100 and 150 mg kg⁻¹ of Z.M. The prepared food was kept in refrigerator until use.

Sampling approach

The fish were sampled at the beginning, midperiod (only for weighing of fish) and at the end of the experiment to determine the values of growth factors and blood parameters throughout the course.

Growth performance

The initial, mid-course and final (60 days) weights of 3 fish in each treatment and replication were applied to evaluate growth performance based on the method of (Kakoolaki et al. 2016) as follows:

$$WG(\%) = Wf - Wi/Wi \times 100$$

: Where, WG was weight gain and mean value of final body weight (Wf) of each group was measure by dividing total fish weight in each tank by number of fish.

$$DGI (\%) = ([(WG \times 100)/(Wi + Wf)/2)]/t$$

: Where, DGI, Wi and Wf were daily growth index, initial and final values of fish weight, respectively.

$$FCR = \frac{\text{feed given (dry weight)}}{\text{body wt.} \times \text{WG (wet weight)}}$$

: Where, FCR was feed conversion ratio.

Blood sampling

Fish were anaesthetized by immersing in the water containing 30 ppm of clove powder. Blood was sampled from the caudal puncture of 3 fish caught at random from each group and replication 8 weeks after the start of the feeding using 1mL heparinated syringe to evaluate hamatocrit and differential white blood count (Abd-El-Rhman 2009) connected to 24-gauge needles.

Blood slides were introduced to Natt-Herrick's solution (1:200) to stained and count RBC (10⁶ mm⁻³) and white blood cells (WBC) (10⁴mm⁻³) (Grant 2015) although the procedure employed to calculate occurrence of these two blood cells was different as above-sentence mentioned. Blood slides stained with Giemsa solution were organized for differential WBC counts (%). Therefore, One hundred leukocytes as well as Lymphocytes, Monocytes, Neutrophil and Eosinophil from each stained slide, were counted using a light microscope.

For hematocrit measurements, heparinized tubes were centrifuged for 3 minutes at $13000 \times g$, and the result solution was then measured

by hematocrit scale reader (Řehulka 2000). Hemoglobin was also measured by photometric assay cyanomethemoglobin approach (Kakoolaki et al., 2016) so that a 20 µl blood sample was drawn from a heparinized capillary tube, mixed in 5.0 ml of cyanhemoglobin reagent (Hycel) and read the absorbance values at 540 nm using a spectrophotometer. The calculation method for other blood parameters is as follows:

$$MCV (fL) = \frac{[(Hct, \%) \times 10]}{(RBC, 10^{6} \text{ per mm}^{3})}$$

$$MCH (pg cell^{-1}) = \frac{[(Hb, g dL^{-1}) \times 10]}{(RBC, 10^{6} \text{per mm}^{3})}$$

$$MCHC (\%) = \frac{[(Hb, g dL^{-1}) \times 100]}{(Hct, \%)}$$

Results

The average weights of rainbow trout affected by Z.M. are listed in Table 1. There was no significant difference (p>0.05) among the fish fed the different levels of Z.M for the mean weight. At the mid time of the experiment, the maximum and minimum mean weights (17.73 \pm 0.48, 13.40 \pm 0.48) were belonged to Z.M. (150 ppm) and control groups (Table 1, Fig. 1). The mean value of fish fed different levels of supplemented diet with Z.M. showed a significant difference (p<0.05) as well as the final weight after 60 days. Accordingly, the maximum and minimum mean weights (23.30 \pm 0.48, 18.83 \pm 0.48) were belonged to Z.M., 100 ppm and control groups (Table 1, Fig. 1).

Table 1. Estimated marginal mean of weigh of the sampled rainbow trout in different levels of *Zataria multiflora* in determined sampling times

Weeks	groups	
		Mean \pm SEM
1	1	4.33 ± 0.48
1	2	4.30 ± 0.48
1	3	4.30 ± 0.48
1	4	4.33 ± 0.48
4	1	13.40 ± 0.48 ^a
4	2	15.03 ± 0.48^{b}
4	3	15.56 ± 0.48^{b}
4	4	$17.73 \pm 0.48^{\circ}$
8	1	18.83 ± 0.48 ^a
8	2	23.30 ± 0.48^{b}
8	3	20.93 ± 0.48^{c}
8	4	19.90 ± 0.48^a

In each sectioned column, no significant difference was observed in similar superscripts.

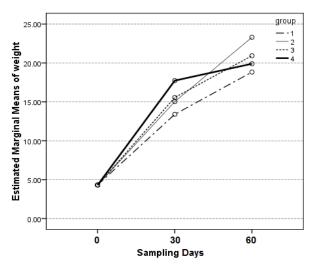


Figure 1. The trend of mean weight of sampled fish in different groups of Zataria multiflora.

Growth parameters and the group comparison among the different level of Z.M. are listed in Table 2. As a result, each of the growth indices containing SGR, FCR and WG were significantly different (p< 0.05) among the Z.M. levels. The maximum value of SGR was observed in group 50 ppm of Z.M. (2.85 ± 0.01) with significant difference (p< 0.05) with control (2.44 ± 0.0). There was no significant difference (p> 0.05) between control and groups 100 and 150 ppm of Z.M. Accordingly, this result showed that FCR value was minimum in control

 (7.20 ± 0.0) with no significant difference (p>0.05) compared with group 50 ppm of Z.M. The maximum value (8.40 ± 0.0) was observed in group of 150 ppm Z.M. with significant difference (p<0.05) compared with control. The result of WG for group 50 ppm of Z.M. (453.86 ± 0.46) showed a significant difference (p<0.05) with those of other groups. The WG of the control group (330.06 ± 2.84) was the least. The values of FCR (Table 3) showed no considerable difference (p>0.05) between levels of control and 50 ppm of Z.M.

Table 2. The average value (Mean \pm SEM) for growth parameters affected by different levels of *Zataria* multiflora

	groups	Mean ± SEM	P value
SGR	Control	2.44±0.00a	0.0
	50 ppm	2.85 ± 0.01^{b}	
	100 ppm	2.64 ± 0.00^{a}	
	150 ppm	2.55 ± 0.00^a	
FCR	Control	7.20 ± 0.0^{a}	0.0
	50 ppm	7.24 ± 0.0^{a}	
	100 ppm	8.20 ± 0.0^b	
	150 ppm	8.40 ± 0.0^b	
WG (%)	Control	330.06 ± 2.84^{a}	0.0
	50 ppm	453.86 ± 0.46^{b}	
	100 ppm	389.90 ± 0.85^{c}	
	150 ppm	362.32 ± 0.24^a	

The variation of the haematological indices affected by different level of Z. multiflora is given in Table 3. Accordingly and based on the WBC and RBC results, there were no significant difference among the grouped Z. multiflora (p= 0.28, p=0.37, respectively). Based on this result, Hemoglobin was experimentally went up (p=0.00)in dose-dependent manner so that the mean values of control was 10.10 ± 0.15 less than those of the 50, 100 and 150 ppm groups, which respectively showed 11.40 \pm 1.15, 13.00 \pm 0.58 and 13.40 \pm 0.26. No significant difference (p > 0.05) was observed between the value of Hb for group 100 and 150 ppm. Hematocrit showed no significant difference among the Z.M. groups so that the least value (25.23 \pm 1.06) was dedicated to the control group with no significance difference (p > 0.05)

with the highest value (26.90 \pm 2.93) in group 50 ppm Z.M. supplemented diet (Table 3). The mean value of MCV showed that the minimum measurement (96.66 \pm 14.76) belonged to Z.M. 150% supplemented diet (table 3) while that of the maximum (104.63 \pm 29.80) presented in group 50 ppm of Z.M. As a result of stated in table 3 there was no significant difference (p>0.05) among the fish fed Z.M. supplemented diet but functionally the MCV values showed considerable differences among the groups. The Values of MCH and MCHC showed no significant difference (p> 0.05) among the different groups affected by different Z.M. The maximum values for MCH and MCHC were 60.885 ± 9.63 (control) and 52.35 ± 3.82 (150 ppm) and those of minimum values were 43.27 \pm 8.33 (50 ppm) and 43.63 ± 5.77 (50 ppm).

Table 3. The average percent (Mean \pm SEM) for haematological parameters affected by different levels of Zataria multiflora

	groups	Mean ± SEM	P value
WBC (10 ⁴)	Control	4.63 ± 0.26	0.28
	50 ppm	5.33 ± 0.49	
	100 ppm	4.39 ± 0.24	
	150 ppm	4.28 ± 0.44	
RBC (10 ⁶)	Control	1.73 ± 0.23	0.37
	50 ppm	2.83 ± 0.52	
	100 ppm	2.80 ± 0.51	
	150 ppm	2.86 ± 0.64	
Hb (g dL ⁻¹)	Control	10.10 ± 0.15^{a}	0.000
	50 ppm	11.40 ± 1.15^{b}	
	100 ppm	13.00 ± 0.58^{c}	
	150 ppm	13.40 ± 0.26^{c}	
Hct (%)	Control	25.23 ± 1.06	0.94
	50 ppm	26.90 ± 2.93	
	100 ppm	26.40 ± 1.90	
	150 ppm	25.83 ± 1.73	
MCV (FL)	Control	150.96 ± 21.44	0.32
	50 ppm	104.63 ± 29.80	
	100 ppm	100.43 ± 18.74	
	150 ppm	96.66 ± 14.76	
MCH (pg cell ⁻¹⁾	Control	60.885 ± 9.63	0.70
	50 ppm	43.27 ± 8.33	
	100 ppm	50.44 ± 11.52	
	150 ppm	51.72 ± 11.51	
MCHC (%)	Control	40.20 ± 2.16	0.18
	50 ppm	43.63 ± 5.77	
	100 ppm	49.54 ± 2.67	
	150 ppm	52.35 ± 3.82	

As a result, Table 4 shows Z. M. with the different levels had no any effect (p = 0.22) on lymphocyte quantity, which the values of the maximum amount occurred in group 100 ppm of Z.M. (78.30 \pm 0.47) was not statistically different (p> 0.05) from the least value of lymphocyte in control (76.20 \pm 0.58). Accordingly, Eosinophil quantity was not

shown any difference (p>0.05) among the different groups, from the least (0.40 ± 0.23) for group of 100 ppm of Z.M. up to those of others. The mean value of neutrophil was statistically increased in group 50 ppm of Z. M. (11.03 ± 0.12) compared with control (9.10 ± 0.41) , which it showed no significant difference (p>0.05) with other groups (Table 4).

Table 4. The average percent (Mean \pm SEM) for differentiated WBC affected by different levels of *Zataria* multiflora

	groups	Mean ± SEM	P value
Lymphocyte %	Control	76.20 ± 0.58	0.22
	50 ppm	76.50 ± 0.75	
	100 ppm	78.30 ± 0.47	
	150 ppm	77.46 ± 0.93	
Monocyte %	Control	14.23 ± 0.29^{a}	0.03
	50 ppm	12.00 ± 0.70^{b}	
	100 ppm	12.10 ± 0.28^b	
	150 ppm	13.36 ± 0.58^a	
Neutrophil %	Control	9.10 ± 0.41 ^a	0.001
	50 ppm	11.03 ± 0.12^{b}	
	100 ppm	9.20 ± 0.17^a	
	150 ppm	8.70 ± 0.23^a	
Eosinophil %	Control	0.46 ± 0.13	0.98
	50 ppm	0.46 ± 0.03	
	100 ppm	0.40 ± 0.23	
	150 ppm	0.46 ± 0.16	

Discussion

According to results of growth indices (Table 2), there was an appropriate effect of 50 ppm Z.M. on specific growth rate in comparison with other levels of Z.M. (0, 100 and 150 ppm). The result of WG confirmed this former finding so that WG (%) of group 50 ppm of Z.M. (453.86 \pm 0.46) was the maximum showed a significant difference (p< 0.05) with those of other groups. As control group showed the least value of WG indicating on the effect of Z.M. with minimum level on growth

while the FCR showed no significant difference in level of 50 ppm of Z.M. compared with control but other groups.

There was no evidence to believe that *Z. multiflora* can experimentally effect on the mean value of WBC and RBC (table 3). This result showed that Hb can increasingly affect due to Z.M. Roche and Bogé (1996) showed that Hb value increases through the environmental factors and chemical intoxication. Grant (2015) presented that Hb

can increase due to induced starvation and variation in water temperature and decrease because of infection (Harikrishnan, Rani & Balasundaram 2003) or as a result of the swelling of RBC as well as weak transfer of hemoglobin from the hematopoeitic tissues. As a result of a study carried out in rainbow trout, the maximum mean value of RBC was observed in group 100 ppm of Z.M. (Akbary, Ghareghani poor & Fereidouni 2015) but in this study, there was no a significant difference was observed among the groups, 50, 100 and 150 ppm of Z.M. Similar to this results, the RBC value in the study was made by Akbary et al. (2015) showed no remarkable difference was shown among the above 3 mentioned treatments. Dislike to this result; Soltani, Sheikhzadeh. Ebrahimzadeh-Mousavi and Zargar (2010) showed an increase trend in WBC affected by Z.M. up to 60 ppm Z.M. supplemented diet while it decreased after increasing Z.M. concentration. our result indicated on a slight increase of Hb due to Z.M. Decreased hemoglobin concentration, RBC and hematocrit values point out that RBCs are being demolished by leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis (Haney, Hursh, Mix & Winton 1992). Presence of immature erythrocytes in outlying blood can be due to infection or younger fish result in decrease of hemoglobin (Walsh & Luer 2004). Choobkar. Kakoolaki. Rezaeimanesh. Mohammadi and Safar Khanloo (2017) presented that 100 ppm of Z.M. can enhance the hemoglobin value in Cyprinus carpio. The packed cell volume (Hematocrit) counts is an

indicator for fish health prognosis (Harikrishnan et al. 2003). The measurement less than 20% in teleosts usually are associated with anemia (Clauss, Dove & Arnold 2008). An upsurge in hematocrit value was stated because of oxygen depletion (Holeton & Randall 1967). Hematocrit increased in dosedependent manner in C. carpio supplemented with Z.M. with no significant (Choobkar et al. 2017). In another study (Ngugi, Oyoo-Okoth, Mugo-Bundi, Orina, Chemoiwa & Aloo 2015) Hct increased from 40% up to 90% in Lobeo victorianus fed with the herb, Urtica dioica after 16 weeks in dosedependent manner. Baba, Acar, Yılmaz, Öntaş and Kesbiç (2017) stated hematocrit was not affected due to argan oil or other dietary lipid sources, Mohseni, Pourali, Kazemi and Bai (2014) showed that increase protein from 30 up to 50 % as supplemented diet can grow Hct of Huso huso juvenile. Juvenile teleosts have notably higher lymphocyte and total leukocyte counts compared with adults (Clauss et al. 2008) against this finding showed no significant changes in juvenile fishes. Young fish and herbal medicines may increase WBC (Grant 2015, Kakoolaki et al. 2016). Simultaneous increase of WBC and neutrophil count maybe indicated on inflammatory or other condition of the fish while rainbow trout is reared in summer or spring or confront with stress (Harikrishnan et al. 2003).

As indicator for specific immunity, this result showed no any effectiveness of Z. M. on the values of Lymphocyte and eosinophil among the different levels of Z. M. Based on

the indication of non-specific immunity, the fish fed supplemented diet with 50 ppm of Z. M. showed more effectiveness for neutrophil. Monocyte values for the fish fed with 50 and 100 ppm of Z.M. showed lower values compared to 150 ppm and control groups.

In the study conducted by Soltani et al. (2010), blood lymphocyte value was increased in fish, C. carpio fed Z. multiflora. They did explain the mechanism involved lymphocyte increasing. Similar to their finding, IgM and neutrophil were significantly increased in the rat fed supplemented diet mixed with Z. multiflora (Dehkordi, Dehkordi, Chaleshtori, Khamesipour & Katsande 2015). This finding is against of this result showed a decrease of lymphocyte count in rainbow trout. Raissy, Fakhrian, Jafarian and Varshoei (2014) showed an increase in value of neutrophil when starlet fish (Acipenser ruthenus) fed Z. multiflora. Our results showed no effectiveness of Z.M. on specific immunity observed in the result of lymphocyte values shown in table 4.

The result of growth indices showed the use of *Zataria multiflora* has no economic efficiency in concentration of 100 and 150 ppm. Accordingly, as a result of differentiate WBC, use of 50 ppm of *Z. multiflora* significantly increases Neutrophil as nonspecific haematological cell. It is concluded that the fish fed with 50 ppm of *Zataria multiflora* increased mean weight of rainbow trout while enhancement of innate immunity significantly was occurred.

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تأثیر آویشن شیرازی بر خصوصیات خونی و رشدی ماهی قزل آلای رنگین کمان

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چكىدە

آویشن شیرازی در مناطق گرم مشرق زمین، ایران، افغانستان و پاکستان مورد کشت قرار می گیرد. هدف این مقاله بر این است که آیا این گیاه بر روی پارامترهای هماتولوژی و رشد ماهی قزل آلای رنگین کمان تأثیر می گذارد. به این منظور ۱۲۰ ماهی انگشت قد سالم با میانگین وزن f/r به صورت تجاری به دست آمد و سپس به منطقه تحقیقاتی واقع در تهران منتقل شد. ماهی در رژیمهای غذایی حاوی f/r به صورت تجاری به دست آمد و سپس به منطقه تحقیقاتی واقع در تهران منتقل شد. ماهی در رژیمهای غذایی حاوی f/r به صورت تجاری به دست آمد و مفته (۶۰ روز) مورد تغذیه قرار گرفتند. نتایج آماری ماهیان به صورت خوراکی f/r وزن بدن) و f/r بار در روز به مدت ۸ هفته f/r هفته f/r وزن متوسط وجود ندارد نشان داد اختلاف معنی داری در بین ماهیهای مختلف تغذیه شده با سطوح مختلف f/r در وزن متوسط وجود ندارد f/r با ختلاف معنی داری در گروه f/r و بدون تفاوت تیمار کنترل f/r شداری در مقایسه با گروه f/r مشاهده گردید. بر این اساس مقدار f/r حداقل در تیمار کنترل f/r با ختلاف معنی داری و بدون تفاوت معنی داری در مقایسه با گروه f/r از آویشن شیرازی بدست آمد. نتیجه رشد وزنی در گروه f/r به ایم (f/r به سخی داری وجود نداشت f/r با سبت به شاهد و سایر تیمارها و مهمچنین f/r افزایش دهد، اما در بین تیمارها اختلاف معنی داری وجود نداشت f/r با سبت به شاهد و سایر تیمارها و معنی داری دمتر از سایرین بود f/r بین تیمارها اختلاف معنی داری وجود نداشت f/r برکس، مقادیر f/r به طور معنی داری کمتر از سایرین بود f/r به به طور معنی داری رخ داده است. افزایش ایمنی ذاتی به طور معنی داری رخ داده است.

کلمات کلیدی: ماهی قزلآلای رنگین کمان، آویشن شیرازی، وزن، شاخصهای هماتولوژی.

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