Iranian Journal of Fisheries Sciences 18(3) 517-530 2019
DOI: 10.22092/ijfs.2019.118350

# Effects of dietary Shirazi thyme (*Zataria multiflora* Boiss) and vitamin E on growth and biochemical parameters in common carp (*Cyprinus carpio*)

**Mohiseni M.[1*]; Sadeghian M.[2]; Nematdust Haghi B.[2]; Bagheri D.[3]**

Received: June 2016 Accepted: April 2017

**Abstract**

Using phytobiotics as fish feed additives has interestingly increased during the recent decade. The aim of the current study was to evaluate the effect of individual and combined levels of thyme and vitamin E in common carp. Juveniles were separately fed four distinct diets including a control diet (commercial diet without any additive), a diet supplemented with 1% ground Shirazi thyme, diet supplemented with 100 mg/kg vitamin E and a mixture of Shirazi thyme and vitamin E (TE) with mentioned concentrations for 45 days. Sampling was done on days 15, 30 and 45 after the feeding trial duration and some growth and plasma biochemical parameters were evaluated. The individual thyme supplementation significantly increased weight gain (%), feed conversion efficiency and decreased feed conversion ratio ($p$<0.05). Plasma enzymes (AST, ALT, ALP and LDH) were not affected in all experimental groups. Compared with the control, triglyceride and glucose were significantly elevated in TE complex at all sampling times. Plasma proteins (total protein, albumin and globulin), cholesterol and creatinine were not altered in all treatments. In conclusion, dietary supplementation with individual 1% Shirazi thyme had no harmful effects on plasma biochemical parameters and increased growth performance of the fish. Moreover, positive effects of thyme on fish growth were more than with vitamin E. Supplementation with a mixture of vitamin E and thyme also displayed no superiority than the individual use of Shirazi thyme in the diet.

**Keywords**: Growth, Biochemical parameter, Phytobiotic, Vitamin E

1-Department of Fisheries Science and Engineering, Lorestan University, Khorramabad, Iran

2-Department of Fisheries, Behbahan Khatam Alanbia University of Technology, Behbahan, Iran

3-Department of Fisheries, Persian Gulf University, Bushehr, Iran

*Corresponding author's Email: Mohiseni@ut.ac.ir



[ DOR: 20.1001.1.15622916.2019.18.3.6.5 ]

## Introduction

There are a large number of feed additives to improve fish growth performance and production (Zaki *et al*., 2012). Some of these additive are hormones and antibiotics which may have undesired harmful effects both directly on fish and indirectly on human beings (Gullu *et al*., 2015). The use of antibiotics and other chemicals for controlling diseases has been criticized for their negative impacts (Pandey *et al.*, 2012). Using herbal products or phytobiotics as drugs in disease management is gaining success because herbal drugs are cost-effective, environmentally friendly and have minimal side effects. Thus the use of phytobiotics is an alternative to antibiotics in fish health management (Pandey *et al.*, 2012). Herbs are not only safe for consumers but also have a significant role in aquaculture (Ahilan *et al.*, 2010). Many studies have proved that herbal additives promoted the growth of fishes and were useful in disease prevention (Khalil *et al.*, 2009; Yilmaz *et al*., 2012; Dorojan *et al.*, 2014; Sonmez *et al*., 2015). Positive aspects of phytobiotics such as growth promoter (El-Dakar *et al.*, 2008), antioxidant (Sönmez *et al.*, 2015), elevation of the nitrogen absorption and simulative effects on enzymatic equipment (Dorojan *et al.*, 2015) have been widely reported.

Thyme is a phytobiotic and has been used since ancient times, in the kitchen, cosmetics and for medicinal purposes. It includes thymol (44-60%) an essential oil having strong antiseptic properties, is rich in antioxidants, potassium, magnesium and vitamins A, C and E. The therapeutic properties of thyme in aquaculture are as an antiseptic, antioxidant, and effective in stimulating digestion etc (Dorojan *et al.*, 2014). Shirazi thyme (*Zataria multiflora* Boiss) is a thyme-like plant belonging to the Lamiaceae family that geographically grows wild only in central and southern parts of Iran, Pakistan and Afghanistan (Hosseinzadeh *et al.*, 2000). It has chemical and pharmacological similarities to common thyme (*Thymus vulgaris*), the well-known and widely investigated medicinal plant (Sajed *et al.*, 2013). The essential oil of Shirazi thyme contains significant quantities of phenolic oxygenated monoterpenes, which have antioxidant, antibacterial and antifungal activities (Ehsani *et al.*, 2014). There are many reports about the antioxidant (Kavoosi *et al.*, 2012), antibacterial (Fazeli *et al.*, 2007; Shakeri *et al.*, 2011) and antifungal (Gandomi *et al.*, 2009) effects of Shirazi thyme. Several studies also indicated the preserving effects of Shirazi thyme on fish fillets and burgers from bacterial contamination (Choobkar *et al.*, 2010; Ehsani *et al.*, 2014). Some studies were also conducted about its immune stimulant and lethal dose. For example, Soltani *et al*. (2013) declared that dietary use of Shirazi thyme essential oil has no effect on serum lysozyme activity in rainbow trout. Sharif Rohani *et al*. (2011) just estimated and reported the lethal concentration of Shirazi thyme in rainbow trout. The rest of the studies emphasized on its positive



[ DOR: 20.1001.1.15622916.2019.18.3.6.5 ]

immunological and disease resistance effects in fish (Soltani *et al*., 2014, 2015). Although much information exists on the positive aspects of Shirazi thyme, literature showed no report about its effects on growth and blood biochemical changes in aquatic animals including fishes. This study, therefore, was conducted to evaluate the effects of Shirazi thyme on growth and plasma biochemical parameters of common carp.

## Materials and methods

*Diet preparation*

Four types of experimental diets were prepared and used for the feeding trial during the experiment. A commercial diet (Naghshin Kermanshah, Iran ) was milled in a feed producer, feed additives were added and mixed thoroughly and finally, the diets re-pelletized with a kitchen grinder using 3mm die (Montero *et al.*, 1999). For the control, a milled commercial diet re-pelletized without any supplementation was used whereas in diets 2 and 3 the commercial diet was supplemented with either 1% of dry feed ground Shirazi thyme (T) (Yılmaz *et al.*, 2013) or 100mg/kg of dry feed vitamin E (E) (Kaushik, 1995; Ortuño *et al.*, 2001). Diet 4 was supplemented with both thyme and vitamin E (TE) at mentioned concentrations. All diets were re-pelletized after supplementation. They remained through natural air flow and after drying were kept in the refrigerator (4˚C).

*Acclimation condition and experimental design*

In autumn 2014, 144 healthy juveniles of common carp (average weight of 34±3 g) were obtained from a Persian fish hatchery (Ahvaz, Iran) and transferred to the Khatam Alanbia, University of Technology (Behbahan). Two weeks before the experiment, juveniles were randomly divided into four groups (with triplicates) and transferred to the separate 300-l tank each containing 12 juveniles and individually equipped with an air stone and heater (25 ±2˚C, pH 7.3). Initially, all fish (in all groups) were fed 3% of their body weight twice daily on a commercial diet for carp. Water exchanging during adaptation period and the main experiment was done at the rate of 30% daily for each tank. After the acclimation period, four mentioned groups were fed only with their own specially prepared diet for 45 days. Feeding rates and culture conditions were similar to the adaptation period. Sampling was done at 15, 30 and 45 days after the start of the experiment. Three fish were caught from each tank (9 fishes per treatment) at each sampling time. Samples were sacrificed by spinal cord dislocation, then blood samples were obtained from the caudal vein using heparinized syringes. The blood was then centrifuged for 10 min at 3,000 rpm and the plasma samples were stored at -30˚C until the analysis. Plasma was used for the testing of biochemical indices. Accordingly, some growth parameters were calculated based on



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the following equations (Xie *et al.*, 2008):

Hepatosomatic index (HSI)=100 (liver weight/fish weight)

Weight gain (WG %)=W2–W1; where W1 and W2 are the initial and final weights, respectively.

Feed conversion ratio (FCR)=feed intake / weight gain

Feed conversion efficiency (FCE)= (weight gain/feed intake)×100

*Blood biochemical analysis*

ALT, AST and LDH activities were determined using UV test techniques (Bergmeyer, 1980). ALP activity was determined by use of the colorimetric assay (Moss and Henderson, 1999). Cholesterol (Abell *et al.*, 1952), triglyceride (Cole *et al.*, 2000), creatinine (Newman and Price, 1998) and glucose (Moss and Henderson, 1999) levels were determined by enzymatic colorimetric test. The total protein was measured using a colorimetric test. The kit works based on the method described by Weichselbaum (1946). Albumin was measured following the method of Wotton and Freeman (1974). Globulin (G) concentration was calculated as the difference between total protein and albumin (El-Demerdash *et al.*, 2004). All mentioned biochemical analysis were measured using a spectrophotometer (Biochrom, England). Commercially diagnostic kits for all the measurements were supplied from Pars Azmun Company (Iran).

*Statistical analysis*

Data generated from the biochemical study were analyzed statistically by analysis of variance (ANOVA) using SPSS statistical software (Version 18). Duncan's multiple range test was used to evaluate the mean difference among individual groups at the 0.05 significance level. Data are presented as a mean±standard error.

**Results**

Fish in all groups successfully accepted the experimental diets and there was no apparent observed mortality during the experiment. Changes in some growth factors during the experimental period have been shown in Fig.1. HSI at all groups did not differ over the experiment. WG% and FCE showed apparent improvement in the T group during the experiment and showed significant differences with respect to the other groups. Accordingly, the least FCR was also recorded for the T group which significantly differed from the control and TE at the end of the experiment (Fig. 1).



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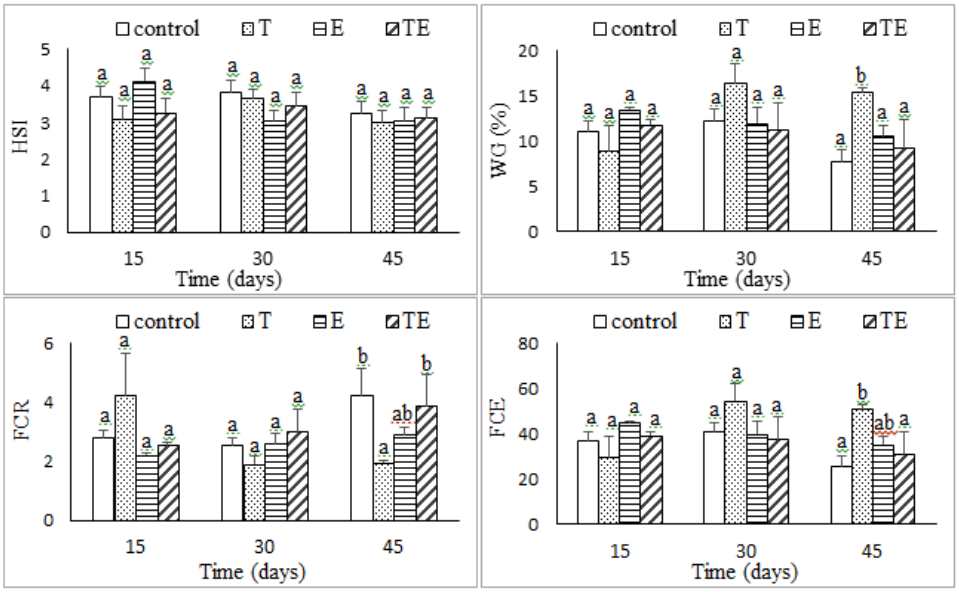


**Figure 1: Effects of experimental diets on liver condition (HSI) and some growth and feed utilization factors (WG%, FCR and FCE) in *Cyprinus carpio* during the experimental period.**

Plasma biochemical changes as a result of thyme and vitamin E supplementation at either individual or complex levels are presented in Tables 1-3. Except those observed on day 15, plasma enzyme activities (AST, ALT, ALP and LDH) were not changed in experimental groups compared with the control. The significantly highest levels of ALT and ALP were recorded in the E group on day 15 (Table 1). Plasma LDH in TE complex also showed higher activity than in the T and E groups ($p$<0.05). There were no variations among all groups on days 15 and 45 for total protein but it was significantly elevated in the fish that were fed with only thyme (T) in their diet on the 30$^{th}$ day (Table 2). Albumin and globulin were also not affected in all experimental groups throughout the experiment. Plasma triglyceride was tended to increase in TE especially on days 15 and 45 of the experiment, Although, significant differences were only seen on the 45$^{th}$ day. Cholesterol showed a slight change in all experimental groups and despite the significant elevation that was recorded with thyme on the 30th day, there was no variation in this factor in all treatments at the end of the experiment. Glucose had the highest level in TE complex throughout the experiment and indicated significant differences with E and T on day 15, control and E on day 30 and T on the 45$^{th}$ day ($p$<0.05). Creatinine also did not exhibit any changes in all treatments during the experiment.



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**Table 1: The effects of thyme and vitamin E supplemented diets on biochemical properties of *Cyprinus carpio* after 15 days trial feeding.**

| | Control | T | E | TE | ANOVA |
|---|---|---|---|---|---|
| AST | 248.1± 98.2 | 397 ± 156.1 | 191.9 ± 86.1 | 184.2 ± 85.8 | n.s. |
| ALT | 46.3 ± 23$^{ab}$ | 35.3 ± 11.8$^{ab}$ | 94.8 ± 33.6$^{b}$ | 24.3 ± 7$^{a}$ | $p$<0.05 |
| ALP | 117.32 ± 11.14$^{ab}$ | 87.46 ± 11.03$^{a}$ | 140.15 ± 20.97$^{b}$ | 85.01 ± 7.59$^{a}$ | $p$<0.05 |
| LDH | 243.12 ± 44.29$^{ab}$ | 201.26 ± 25.94$^{a}$ | 188.09 ± 39.36$^{a}$ | 286.76 ± 77.34$^{b}$ | $p$<0.05 |
| Total protein | 2.48 ± 0.45 | 2.06 ± 0.1 | 2.73 ± 0.17 | 2.28 ± 0.77 | n.s. |
| Albumin | 1.46 ± 0.66 | 1.06 ± 0.42 | 1.44 ± 0.62 | 1.27 ± 0.08 | n.s. |
| Globulin | 1.02 ± 0.8 | 1 ± 0.49 | 1.29 ± 0.6 | 1.01 ± 0.8 | n.s. |
| Triglyceride | 298.45 ± 44.21$^{ab}$ | 231.46 ± 21.56$^{a}$ | 226.95 ± 33.07$^{a}$ | 432.82 ± 71.68$^{b}$ | P<0.05 |
| Cholesterol | 132.74 ± 12.52 | 142.66 ± 16.03 | 115.24 ± 6.09 | 115.55 ± 7.93 | n.s. |
| Glucose | 82.77 ± 18.6$^{ab}$ | 73.2 ± 11.16$^{a}$ | 65.83 ± 9.38$^{a}$ | 103.89 ± 18.9$^{b}$ | P<0.05 |
| Creatinine | 0.9 ± 0.16 | 1.37 ± 0.18 | 1.19 ± 0.12 | 0.92 ± 0.06 | n.s. |

Values in rows with the dissimilar superscript are significantly different from each other (ANOVA, Duncan test)
ns = no significant differences found.

**Table 2: The effects of thyme and vitamin E supplemented diets on biochemical properties of *Cyprinus carpio* after 30 days trial feeding.**

| | Control | T | E | TE | ANOVA |
|---|---|---|---|---|---|
| AST | 452.1 ± 222.2 | 154.4 ± 51 | 249.2 ± 56.8 | 169.8 ± 117.5 | n.s. |
| ALT | 18.7 ± 7.7 | 35.3 ± 15.1 | 15.4 ± 8.1 | 46.3 ± 12.2 | n.s. |
| ALP | 107.22 ± 13.86 | 62.03 ± 9.49 | 78.42 ± 5.02 | 67.24 ± 15 | n.s. |
| LDH | 128.24 ± 37.02 | 179.89 ± 44.29 | 155.85 ± 35.8 | 152.28 ± 29.29 | n.s. |
| Total protein | 1.91 ± 0.56$^{a}$ | 3.42 ± 0.62$^{b}$ | 1.95 ± 0.8$^{a}$ | 2.43 ± 1.41$^{a}$ | $p$<0.01 |
| Albumin | 1.19 ± 0.26 | 2.44 ± 0.51 | 0.88 ± 0.54 | 1.15 ± 1.05 | n.s. |
| Globulin | 0.72 ± 0.4 | 0.98 ± 0.5 | 1.07 ± 0.4 | 1.28 ± 0.55 | n.s. |
| Triglyceride | 367.02 ± 80.1 | 280.67 ± 29.28 | 295.13 ± 33.28 | 353.36 ± 65.65 | n.s. |
| Cholesterol | 118.96 ± 10.94$^{a}$ | 206.94 ± 19.87$^{b}$ | 168.21 ± 19.35$^{ab}$ | 157.99 ± 25.02$^{ab}$ | $p$<0.05 |
| Glucose | 81.45 ± 25.33$^{a}$ | 91.76 ± 15.89$^{ab}$ | 74.04 ± 8.33$^{a}$ | 133.07 ± 6.51$^{b}$ | $p$<0.01 |
| Creatinine | 1.06 ± 0.1 | 1.3 ± 0.2 | 1.01 ± 0.09 | 1.03 ± 0.1 | n.s. |

Values in rows with the dissimilar superscript are significantly different from each other (ANOVA, Duncan test)
ns = no significant differences found.



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**Table 3: The effects of thyme and vitamin E supplemented diets on biochemical properties of *Cyprinus carpio* after 45 days trial feeding.**

| | Control | T | E | TE | ANOVA |
|---|---|---|---|---|---|
| AST | 184.2 ± 81.7 | 115.1 ± 87.9 | 225 ± 91.1 | 119.5 ± 46.2 | n.s. |
| ALT | 62.1 ± 50 | 62.8 ± 21.2 | 97 ± 30 | 84.9 ± 26.9 | n.s. |
| ALP | 139.07 ± 26.18 | 221.32 ± 22.02 | 174 ± 14.06 | 157.91 ± 19.6 | n.s. |
| LDH | 255.59 ± 20.06 | 111.32 ± 42.28 | 244.9 ± 21.67 | 216.4 ± 82.97 | n.s. |
| Total protein | 1.89 ± 0.16 | 1.44 ± 0.08 | 1.78 ± 0.14 | 1.72 ± 0.2 | n.s |
| Albumin | 0.95 ± 0.34 | 0.89 ± 0.21 | 1.06 ± 0.61 | 0.88 ± 0.2 | n.s. |
| Globulin | 0.94 ± 0.35 | 0.55 ± 0.63 | 0.72 ± 0.33 | 0.84 ± 0.21 | n.s. |
| Triglyceride | $247.64 \pm 22.2^{a}$ | $228.88 \pm 108.29^{a}$ | $311.05 \pm 31.53^{a}$ | $453.77 \pm 47.29^{b}$ | $p<0.05$ |
| Cholesterol | 153.19 ± 11.01 | 171.93 ± 29.93 | 180.3 ± 25.6 | 117.72 ± 14.56 | n.s |
| Glucose | $86.13 \pm 5.14^{ab}$ | $71.15 \pm 12.25^{a}$ | $116.53 \pm 18.65^{ab}$ | $122.79 \pm 5.15^{b}$ | $p<0.05$ |
| Creatinine | 0.38 ± 0.09 | 0.27 ± 0.11 | 0.31 ± 0.11 | 0.29 ± 0.13 | n.s. |

Values in rows with the dissimilar superscript are significantly different from each other (ANOVA, Duncan test)
ns = no significant differences found.

## Discussion

The present study demonstrated that supplementation of the diet with Shirazi thyme not only showed no negative effects on plasma biochemical parameters but also, on the other hand, led to significant improvement in some growth indices in *C. carpio*. Results showed that WG%, FCE and FCR were significantly improved in the individual thyme (T) group (Fig. 1). Our finding are in agreement with Dorojan *et al.* (2014) who reported growth improvement in stellate sturgeon as a result of dietary thyme supplementation. Similarly, Zaki *et al.* (2012) evaluated the effects of several phytobiotics in two doses (1% and 2%) in *Oreochromis niloticus*. They concluded that dietary supplementation of 1% thyme had a positive effect on growth performance parameters. Similar conclusions have been reported by feed administration of sage, mint and thyme oil in rainbow trout (Sonmez *et al*., 2015). Phytobiotics contribute to improving the defense mechanism of fish and hence show protection against stressful conditions and consequently can elevate the fish physiological fitness (Xie *et al.*, 2008). Shirazi thyme is mainly composed of monoterpene and aromatic compounds that have antibacterial, antiviral, antifungal and antioxidant activities. Both plant essential oil and water extracts contain considerable amounts of carvacrol, thymol, p-cymene and flavonoids (Sengul *et al*., 2008; Kavoosi *et al.*, 2012). The antioxidant activity of these compounds has been reported in several investigations (Miura *et al.*, 2002; Hamzawy *et al.*, 2012; Kavoosi *et al.*, 2012; Sajed *et al*., 2013). These antioxidants can promote liver health and function and therefore induce more


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normal metabolism, consequently improving animal growth performance (Miura *et al.*, 2002). On the other hand, thymol has a potentially inductive effect on the secretion of pancreatic enzymes and thereby can improve FCR (Lee *et al.*, 2003). Moreover, active compounds in thyme oil can improve the digestibility of the feed (Sengul *et al.*, 2008). These results emphasized that essential oils of phytobiotics can stimulate enzyme activity and improve the absorption of the feed.

Blood enzymes are widely used as biochemical indicators for detecting stress or disease conditions (Adham *et al.*, 1999; Mohiseni *et al.*, 2016). It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of cellular damage (Fırat *et al.*, 2011). Except for the recorded data on the 15$^{th}$ day, results from the current study showed no significant changes in plasma enzyme activities. Since, the enzyme activities were considerably moderated in further sampling times, the observed enzyme alteration on day 15 may be due to the abrupt change in diet composition. Unaffected enzyme activity in all experimental groups could reflect the normal function of hepatic cells (Roncarati *et al.*, 2006). There are many reports confirming that using phytobiotics in fish diet produced no liver disorder (Roncarati *et al.*, 2006; Ji *et al.*, 2007; Oskoii *et al.*, 2012; Fereidouni *et al.*, 2015; Soleimani *et al*., 2016). Several reports, on the other hand, also emphasized on their positive effects on the improvement of liver function (Hamzawy *et al.*, 2012; Hernández *et al.*, 2015).

Main blood lipid components including triglycerides and cholesterol can be affected by diet and also stress in fish (Wiegertjes *et al.*, 1996). The cholesterol had slight changes during the experiment and the significantly higher level was only recorded on the 30$^{th}$ day for T compared with the control group. Sengul *et al*. (2008) found that compounds of thyme oil did not affect plasma cholesterol levels in either high or low cholesterol in broilers. They hypothesized that thyme had no hypocholesterolaemic capability. Triglyceride, on the other hand, indicated more variation particularly in the TE complex as they revealed significant increase compared to the other groups. Ji *et al.* (2007) reported that using dietary herbs mixture did not stimulate any change in plasma triglyceride in Japanese flounder. Similar results have been reported using thyme powder in the diet of laying hens (Mansoub, 2011). Using a mixture of vitamin E and thyme seems to lead to an increase in triglyceride in TE group. As described previously, thymol and carvacrol are two main components of Shirazi thyme. Yilmaz and Ergün (2015) found that carvacrol can increase dietary lipids emulsification and hence can facilitate their absorption into the blood. As the vitamin E is a fat soluble substance, this effect may be due to its more additional absorption. According to this hypothesis, glucose was also significantly elevated in the TE group. This may reflect the negative



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interaction between thyme compounds and vitamin E, although the other biochemical and growth parameters had not confirmed the undesirable effects of TE complex.

Total protein, albumin and globulin were not affected in our experiment. Exceptionally, total protein in T group was significantly increased on the 30$^{th}$ day compared with the other groups as well as the control. Gulec *et al.* (2013) reported that serum total protein and albumin in rainbow trout fed dietary thyme increased after experimental infection by *Yersinia ruckeri*. On the other hand in agreement with our findings, unaffected total protein, albumin and globulin as a result of carvacrol administration in the diet of rainbow trout were reported in the similar study (Yilmaz and Ergün, 2015). Creatinine was also not affected in the present study suggesting that dietary supplementation of Shirazi thyme had no stressful effect on the renal tissues.

Generally, it can be concluded that dietary supplementation with 1% Shirazi thyme not only showed no adverse effects on plasma biochemical parameters but also led to significant improvement in growth indices. Moreover, positive effects in growth performance for thyme was more than in the vitamin E supplemented diet. The mixture of vitamin E and thyme showed no priority to individual thyme in diet supplementation, especially in terms of growth performance. Based on the obtained results using dietary 1 percent Shirazi thyme would help to elevate health and growth in common carp.

**Acknowledgments**

This study was supported by Behbahan Khatam Alanbia University of Technology. The authors would like to sincerely thank the staff in the laboratories in natural resources faculty for their cooperation in sample analyses.

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