

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

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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

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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

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 ($25 \pm 2^\circ\text{C}$, 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

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

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

Weight gain (WG %)= $\frac{W2-W1}{W1} \times 100$; where W1 and W2 are the initial and final weights, respectively.

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

Feed conversion efficiency (FCE)= $\frac{\text{weight gain}}{\text{feed intake}} \times 100$

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 \pm 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).

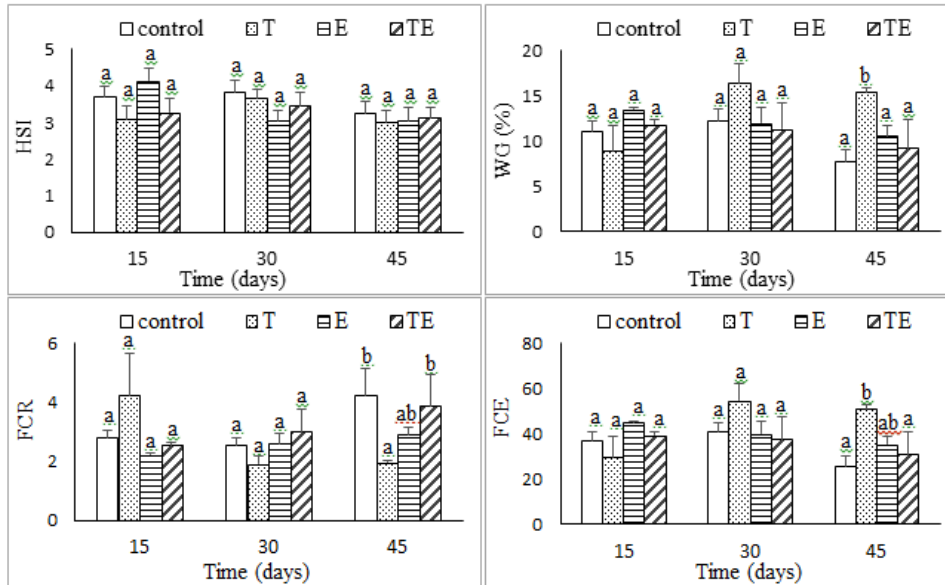


Figure 1: Effects of experimental diets on liver condition (HSI) and some growth and feed utilization factors (WG%, FCR and FCE) in *Cyprinus carpio* during the experimental period.

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 30th 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 45th 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 45th day ($p < 0.05$). Creatinine also did not exhibit any changes in all treatments during the experiment.

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	<i>p</i> <0.05
ALP	117.32 ± 11.14 ^{ab}	87.46 ± 11.03 ^a	140.15 ± 20.97 ^b	85.01 ± 7.59 ^a	<i>p</i> <0.05
LDH	243.12 ± 44.29 ^{ab}	201.26 ± 25.94 ^a	188.09 ± 39.36 ^a	286.76 ± 77.34 ^b	<i>p</i> <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	<i>P</i> <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	<i>P</i> <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	<i>p</i> <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}	<i>p</i> <0.05
Glucose	81.45 ± 25.33 ^a	91.76 ± 15.89 ^{ab}	74.04 ± 8.33 ^a	133.07 ± 6.51 ^b	<i>p</i> <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.

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 ± 22.2 ^a	228.88 ± 108.29 ^a	311.05 ± 31.53 ^a	453.77 ± 47.29 ^b	<i>p</i> <0.05
Cholesterol	153.19 ± 11.01	171.93 ± 29.93	180.3 ± 25.6	117.72 ± 14.56	n.s.
Glucose	86.13 ± 5.14 ^{ab}	71.15 ± 12.25 ^a	116.53 ± 18.65 ^{ab}	122.79 ± 5.15 ^b	<i>p</i> <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 findings 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; Kavooosi *et al.*, 2012). The antioxidant activity of these compounds has been reported in several investigations (Miura *et al.*, 2002; Hamzawy *et al.*, 2012; Kavooosi *et al.*, 2012; Sajed *et al.*, 2013). These antioxidants can promote liver health and function and therefore induce more

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 (Firat *et al.*, 2011). Except for the recorded data on the 15th 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; Oskoi *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 30th 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

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 30th 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.

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References

- Abell, L.L., Levy, B.B., Brodie, B.B. and Kendall, F.E., 1952.** A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *Journal of Biological Chemistry*, 195, 357-366.
- Adham, K., Hassan, I., Taha, N. and Amin, T., 1999.** Impact of hazardous exposure to metals in the Nile and Delta lakes on the catfish, *Clarias lazera*. *Environmental Monitoring and Assessment*, 54(2), 107-124.
- Ahilan, B., Nithiyapriyatharshini, A. and Ravaneshwaran, K., 2010.** Influence of certain herbal additives on the growth, survival and disease resistance of goldfish, *Carassius auratus* (Linnaeus). *Tamilnadu Journal of Veterinary and Animal Science*, 6(1), 5-11.
- Bergmeyer, H., 1980.** IFCC methods for the measurement of catalytic concentrations of enzymes: Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6. 1.2). *Journal of clinical chemistry and clinical biochemistry*, 105(1), 147-154.

- Choobkar, N., Soltani, M., Ebrahimzadeh Mousavi, H., Akhonzadeh Basti, A. and Matinfar, A., 2010.** Effect of *Zataria multiflora* Boiss essential oil on the growth of *Staphylococcus aureus* in the light salted fillets of silver carp (*Hypophthalmichthys molitrix*). *Iranian Journal of Fisheries Sciences*, 9(3), 352-359.
- Cole, T.G., Klotzsch, S.G. and McNamara, J.R., 2000.** Measurement of triglyceride concentration. In: Rifai, N., Warnick, G. & Dominiczak, M. (eds.) Handbook of lipoprotein testing. Washington: AACC Press. page 13-25
- Dorojan, O.G., Placinta, S. and Petrea, S., 2014.** The influence of some phytochemicals (thyme, seabuckthorn) on growth performance of stellate sturgeon (*A. stellatus*, Pallas, 1771) in an industrial recirculating aquaculture system. *Scientific Papers Animal Science and Biotechnologies*, 47(1), 205-210.
- Dorojan, O.G.V., Cristea, V., Crețu, M., Dediu, L., Docan, A.I. and Coadă, M.T., 2015.** The effect of thyme (*Thymus vulgaris*) and vitamin E on the *Acipenser stellatus* juvenile welfare, reared in a recirculating aquaculture. *AACL Bioflux*, 8(2), 150-158.
- Ehsani, A., Jasour, M. S., Hashemi, M., Mehryar, L. and Khodayari, M., 2014.** *Zataria multiflora* Boiss essential oil and sodium acetate: how they affect shelf life of vacuum-packaged trout burgers. *International Journal of Food Science and Technology*, 49(4), 1055-1062.
- El-Dakar, A., Hassanien, G., Gad, S. and Sakr, S., 2008.** Use of dried basil leaves as a feeding attractant for hybrid Tilapia, *Oreochromis niloticus* X *Oreochromis aureus*, fingerlings. *Mediterranean Aquaculture Journal*, 1(1), 35-44.
- El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S. and Baghdadi, H.H., 2004.** Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β -carotene. *Food and Chemical Toxicology*, 42(10), 1563-1571.
- Fazeli, M.R., Amin, G., Attari, M.M. A., Ashtiani, H., Jamalifar, H. and Samadi, N., 2007.** Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Control*, 18(6), 646-649.
- Fereidouni, M.S., Akbary, P. and Soltanian, S., 2015.** Survival rate and biochemical parameters in *Mugil cephalus* (Linnaeus, 1758) larvae fed garlic (*Allium sativum* L.) extract. *American Journal of Molecular Biology*, 5(1), 7-12.
- Firat, Ö., Cogun, H. Y., Yüzereroğlu, T. A., Gök, G., Firat, Ö., Kargin, F. and Kötemen, Y., 2011.** A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. *Fish*

- Physiology and Biochemistry*, 37(3), 657-666.
- Gandomi, H., Misaghi, A., Basti, A. A., Bokaei, S., Khosravi, A., Abbasifar, A. and Javan, A.J., 2009.** Effect of *Zataria multiflora* Boiss. essential oil on growth and aflatoxin formation by *Aspergillus flavus* in culture media and cheese. *Food and Chemical Toxicology*, 47(10), 2397-2400.
- Gulec, A.K., Danabas, D., Ural, M., Seker, E., Arslan, A. and Serdar, O., 2013.** Effect of mixed use of thyme and fennel oils on biochemical properties and electrolytes in rainbow trout as a response to *Yersinia ruckeri* infection. *Acta Veterinaria Brno*, 82(3), 297-302.
- Güllü, K., Acar, Ü., Kesbiç, O.S., Yılmaz, S., Ağdamar, S., Ergün, S. and Türker, A., 2015.** Beneficial effects of Oral Allspice, *Pimenta dioica* powder supplementation on the hemato-immunological and serum biochemical responses of *Oreochromis mossambicus*. *Aquaculture Research*, 47(9), 2697-2704.
- Hamzawy, M.A., El-Denshary, E., Hassan, N.S., Manna, F. and Abdel-Wahhab, M.A., 2012.** Antioxidant and hepatorenoprotective effect of thyme *vulgaris* extract in rats during aflatoxicosis. *Global Journal Pharmacology*, 6(2), 106-117.
- Hernández, A., García, B.G., Caballero, M. and Hernández, M., 2015.** Preliminary insights into the incorporation of rosemary extract (*Rosmarinus officinalis* L.) in fish feed: influence on performance and physiology of gilthead seabream (*Sparus aurata*). *Fish Physiology and Biochemistry*, 41(4), 1-10.
- Hosseinzadeh, H., Ramezani, M. and Salmani, G.A., 2000.** Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. *Journal of Ethnopharmacology*, 73(3), 379-385.
- Ji, S.C., Jeong, G.S., Im, G.S., Lee, S.W., Yoo, J.H. and Takii, K., 2007.** Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. *Fisheries Science*, 73(1), 70-76.
- Kaushik, S., 1995.** Nutrient requirements, supply and utilization in the context of carp culture. *Aquaculture*, 129(1), 225-241.
- Kavoosi, G., Teixeira da Silva, J.A. and Saharkhiz, M.J., 2012.** Inhibitory effects of *Zataria multiflora* essential oil and its main components on nitric oxide and hydrogen peroxide production in lipopolysaccharide-stimulated macrophages. *Journal of Pharmacy and Pharmacology*, 64(10), 1491-1500.
- Khalil, F., Farrag, F. and Mehrim, A., 2009.** Using *Majorana hortensis* against contamination of mono-sex Nile tilapia *Oreochromis niloticus* diet by lead oxide. 2nd global fisheries and aquaculture research conference, Cairo International Convention Center, October 2009 Egypt. *Abbassa International*

- Journal for Aquaculture*, 9(1), 407-428.
- Lee, K.-W., Everts, H., Kappert, H., Frehner, M., Losa, R. and Beynen, A., 2003.** Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British poultry science*, 44(3), 450-457.
- Mansoub, N.H., 2011.** Assessment on effect of thyme on egg quality and blood parameters of laying hens. *Annals of Biological Research*, 2(4), 417-422.
- Miura, K., Kikuzaki, H. and Nakatani, N., 2002.** Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *Journal of Agricultural and Food Chemistry*, 50(7), 1845-1851.
- Mohiseni, M., Asayesh, S., Shafiee Bazarnioie, S., Mohseni, F., Moradi, N., Matouri, M., and Mirzaee, N., 2016.** Biochemical alteration induced by cadmium and lead in common carp via an experimental food chain. *Iranian Journal of Toxicology*, 10(4), 25-32.
- Montero, D., Marrero, M., Izquierdo, M., Robaina, L., Vergara, J. and Tort, L., 1999.** Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. *Aquaculture*, 171(3), 269-278.
- Moss, D. and Henderson, A., 1999.** Clinical enzymology-in TITETZ textbook of clinical chemistry. *In:* Burtis, C. and Ashwood, E. (eds.) Textbook of clinical chemistry. Philadelphia: WB Saunders Company. 671-673.
- Newman, D.J. and Price, C., 1998.** Renal function and nitrogen metabolites. *In:* Burtis, C.A. and Ashwood, E. (eds.) *Tietz Textbook of Clinical Chemistry*. 3rd ed. ed. Philadelphia: WB Saunders Company. 1204-1270.
- Ortuño, J., Cuesta, A., Esteban, M.A. and Meseguer, J., 2001.** Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparus aurata* L.) innate immune system. *Veterinary Immunology and Immunopathology*, 79(3), 167-180.
- Oskoi, S.B., Kohyani, A.T., Parseh, A., Salati, A.P. and Sadeghi, E., 2012.** Effects of dietary administration of *Echinacea purpurea* on growth indices and biochemical and hematological indices in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Fish Physiology and Biochemistry*, 38(4), 1029-1034.
- Pandey, G., Madhuri, S. and Mandloi, A., 2012.** Medicinal plants useful in fish diseases. *Plant Archives*, 12(1), 1-4.
- Roncarati, A., Melotti, P., Dees, A., Mordenti, O. and Angellotti, L., 2006.** Welfare status of cultured seabass (*Dicentrarchus labrax* L.) and seabream (*Sparus aurata* L.) assessed by blood parameters and tissue characteristics. *Journal of Applied Ichthyology*, 22(3), 225-234.

- Sajed, H., Sahebkar, A. and Iranshahi, M., 2013.** *Zataria multiflora* Boiss.(Shirazi thyme)—an ancient condiment with modern pharmaceutical uses. *Journal of Ethnopharmacology*, 145(3), 686-698.
- Sengül, T., Yurtseven, S., Cetin, M., Kocyigit, A. and Sögüt, B., 2008.** Effect of thyme (*T. vulgaris*) extracts on fattening performance, some blood parameters, oxidative stress and DNA damage in Japanese quails. *Journal of Animal and Feed Sciences*, 17(4), 608-620.
- Shakeri, M.S., Shahidi, F., Beiraghi-Toosi, S. and Bahrami, A., 2011.** Antimicrobial activity of *Zataria multiflora* Boiss. essential oil incorporated with whey protein based films on pathogenic and probiotic bacteria. *International Journal of Food Science and Technology*, 46(3), 549-554.
- Sharif Rohani, M., Haghghi, M. and Assaeian, H., 2011.** The lethal concentration (LC50) of *Zataria multiflora* essential oil in fries of rainbow trout (*Oncorhynchus mykiss*). *Iranian Scientific Fisheries Journal*, 20(2), 89-96.
- Soleimany, V., Banaee, M., Mohiseni, M., Nematdoost Hagi, B. and Mousavi Dehmourdi, L., 2016.** Evaluation of pre-clinical safety and toxicology of *Althaea officinalis* extracts as naturopathic medicine for common carp (*Cyprinus carpio*). *Iranian Journal of Fisheries Sciences*, 15(2), 613-629.
- Soltani, M., Zarifmanesh, T. and Zorriehzaha, S., 2013.** Effect of *Zataria multiflora* essential oils on rainbow trout (*Oncorhynchus mykiss*) complement component activity and lysozyme. *Iranian Scientific Fisheries Journal*, 21(4), 13-23.
- Soltani, M., Mohamadian, S., Ebrahimzahe-Mousavi, H.A., Mirzargar, S., Taheri-Mirghaed, A., Rouholahi, S. and Ghodratnama, M., 2014.** Shirazi thyme (*Zataria multiflora*) essential oil suppresses the expression of the epsD capsule gene in *Lactococcus garvieae*, the cause of lactococcosis in farmed fish. *Aquaculture*, 433, 143-147.
- Soltani, M., Mohamadian, S., Rouholahi, S., Soltani, E. and Rezvani, S., 2015.** Shirazi thyme (*Zataria multiflora*) essential oil suppresses the expression of Pava and Hly genes in *Lactococcus garvieae*, the causative agent of lactococcosis in farmed fish. *Aquaculture*, 442, 74-77.
- Sönmez, A.Y., Bilen, S., Alak, G., Hisar, O., Yanık, T. and Biswas, G., 2015.** Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. *Fish Physiology and Biochemistry*, 41(1), 165-175.
- Weichselbaum, T.E., 1946.** An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *American Journal of Clinical Pathology*, 10, 40-49.
- Wiegertjes, G.F., Stet, R.M., Parmentier, H.K. and van**

- Muiswinkel, W.B., 1996.** Immunogenetics of disease resistance in fish: a comparative approach. *Developmental and Comparative Immunology*, 20(6), 365-381.
- Wotton, I. and Freeman, H., 1974.** Microanalysis in medicinal biochemistry. Churchill Livingstone, Wotton, 1230 P.
- Xie, J., Liu, B., Zhou, Q., Su, Y., He, Y., Pan, L., Ge, X. and Xu, P., 2008.** Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp. *Cyprinus carpio Aquaculture*, 281(1), 5-11.
- Yilmaz, E. and Ergün, S., 2015.** Influence of carvacrol on the growth performance, hematological, non-specific immune and serum biochemistry parameters in rainbow trout (*Oncorhynchus mykiss*). *Food and Nutrition Sciences*, 6(5), 523-534.
- Yilmaz, S., Ergün, S. and Çelik, E.Ş., 2013.** Effect of dietary herbal supplements on some physiological conditions of sea bass *Dicentrarchus labrax*. *Journal of Aquatic Animal Health*, 25(2), 98-103.
- Yilmaz, S., Ergün, S. and Çelik, E.Ş., 2012.** Effects of herbal supplements on growth performance of sea bass (*Dicentrarchus labrax*): Change in body composition and some blood parameters. *Journal of BioScience and Biotechnology*, 1(3), 217-222.
- Zaki, M., Labib, E., Nour, A., Tonsy, H. and Mahmoud, S., 2012.** Effect some medicinal plants diets on mono sex Nile tilapia (*Oreochromis niloticus*), growth performance, feed utilization and physiological parameters. *APCBEE Procedia*, 4, 220-227.