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Growth performance, hepatic function parameters, histological changes of Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and oxidative stability index of feed following dietary administration of Mix-Oil

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Abstract

The main objective of this study was to evaluate the effects of dietary supplementation of Mix-Oil (including: *Thymus vulgaris* (%0.5), *Origanum vulgare* (2%) and *Eucalyptus* spp. (2.5%) essential oils) on growth performance, hepatic function parameters, tissue changes of rainbow trout and oxidative stability index of trout feed. Four groups of trout fish (with average weight 31.0±0.1 g) were fed with four diets containing: 0, 50, 200 and 400 ppm of Mix-Oil for 8 weeks. SGR, ADG, CF, TGC, DGC, RGR and BWI were significantly higher and also FCR significantly lower were observed in 200 and 400 ppm groups compared to 50 ppm and control groups (p<0.05). Significant increase in PER, LER, FCE and RFI in fish fed with 200 and 400 ppm Mix-Oil were observed compared with control and 50 ppm groups (p<0.05), while, no significant differences having been observed among different treatments in terms of AST and ALT level (p>0.05). An increase in the number of hematopoietic cells in the kidney and significant increase in the length of the intestinal villous and the number of related epithelium cells having been seen in the 400 ppm treatment group compared to the other studied treatment groups (p<0.05). By increasing the concentration of Mix-Oil, trout feed resistance has increased against oxidation. Although, the observed increments were only statistically significant in the 200 and 400 ppm Mix-Oil (p<0.05) compared to 50 ppm and control groups. The results suggested that dietary administration of Mix-Oil at the level of 400 ppm could improve growth performance of rainbow trout as well as increase the feed stability.

Keywords: Mix-Oil, Growth performance, *Oncorhynchus mykiss*, Tissue changes, Oxidative stability index of feed

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Introduction

Fish products as a valuable source of protein represent world's best source of high-quality protein with important micronutrients for good health. Since 1990, fish capture has fixed at about 90 million tons in the world, and after that aquaculture has been replaced as the main source for increased growing fish supply (FAO, 2016). On the other hand, the infectious diseases has emerged as a main obstacle to aquaculture industries worldwide, inducing hundreds of millions of dollars economic losses annually (Dadar *et al*., 2016; Soleimany *et al*., 2016).

So, antibiotics have been used widely in modern aquaculture to prevent or treat the bacterial diseases in farmed fish. Therefore, antibiotics consumption has been raised the concerns about cross-resistance as well as the multiple resistances (Adel *et al*., 2017). For these reasons, the development of bacterial resistance has emerged the critical demand to evaluate new antibacterial agents. Therefore, in various diseases, immune stimulant effects of different medicinal plant extracts have been evaluated on fish, for preventing or treating of disease or growth promoter abilities (Adel *et al*., 2015; Hoseinifar *et al*., 2017). Some herbal medicines, including *Thymus vulgaris*, *Origanum vulgare* and *Eucalyptus sp*. that are commonly identified as safe substances (GRAS) (http://www.cfsan.fda.gov/ _ dms/eafus.html) are known for inducers of growth performance, immune system, blood factors and intestinal selected bacterial population (Ertas *et al*., 2005; Zheng *et al*., 2015). High antioxidant and antimicrobial effects revealed by *T. vulgaris* extracts because of high rich source of thymol (Marino *et al*., 1999; Dorman and Deans 2000; Akbarinia and Mirza 2008; Rota *et al*., 2008). Also, several studies have been reported *O. vulgare* with radical-scavenging, antimicrobial, antioxidant and cytotoxic activities properties due to high phenolic content *(Carvacrol* and *thymol*), and two monoterpene hydrocarbons of γ-terpinene, and p-cymene (Şahin *et al*., 2004; Chun *et al*., 2005; Faleiro *et al*., 2005). Moreover, *Eucalyptus* sp. leaves explored as natural antibiotic for the treatment of numerous infectious diseases (Ghalem and Mohamed, 2008; Elaissi *et al*., 2011; Mulyaningsih *et al*., 2011; Bachir and Benali 2012). The main antibacterial components of *Eucalyptus species* were 1,8-cineole followed byα-pinene, p-cymene, borneol, cryptone, spathulenol, viridiflorol and limonene (Elaissi *et al*., 2011). Recently, some studies analyzed these herbal medicines to analysis potent alternatives to antibiotics as therapeutic and prophylactic agents in aquaculture systems (De Rosa *et al*., 1994; Foysal *et al*., 2011; Salehi *et al*., 2016), but there are no reports that evaluate co-administration of *T. vulgaris*, *O. vulgare* and *Eucalyptus* sp. extracts on growth indices, liver enzymes and tissue changes of rainbow trout (*O. mykiss*). For example, it has been reported that dietary thyme had a various effect since used as an oil or herb on body mass and weight gain in chickens from 7 to 28 days of age

(Cross *et al*., 2007). However, there is only rare data about whether the herb extracts would have the growth promoting effects in fish.

Therfore, the main objectives of this study were to study the effects of dietary supplementation of Mix Oil (including: *Thymus vulgaris*, *O. vulgare* and *Eucalyptus* spp essential oils) on growth performance, hepatic function parameters, tissue changes of rainbow trout and oxidative stability index of trout feed.

Materials and methods

Animal and experimental conditions

A total of 840 rainbow trout juveniles with an average weight 31.0±0.1 g (mean±SE) were obtained from a commercial fish farm in Mazandaran province, Iran and transferred to the Chalus farm in November 2017 (Sari, Iran). The fish having been acclimatized to laboratory rearing conditions for 2 weeks and have been provided with a commercial diet 3 times per day at 3 % body weight (Table 1). After the acclimation period, apparently healthy fish have been selected and were randomly divided into 12 3000-L cement ponds with a stocking density of 70 fish per tank. The physicochemical properties of water during acclimation and actual experiment were maintained as follows: dissolved oxygen at 9.26±0.1 mg L^{-1}, pH at 7.34±0.7, temperature at 16.0±1.2^{o}C (mean±SE). The fish were subjected to a 16L: 8D photoperiod regime.

Table 1: **Proximate composition of basal diet.**

Proximate composition (% dry weight)	
Crude protein	41
Crude lipid	12
Ash	8.2
Fiber	2.3
Moisture	4.76
NFE [a]	15.3
Energy (MJ kg^{-1}) [b]	20.6

[a]Nitrogen-free extracts (NFE)=100- (crude protein+crude lipid +ash)
[b]Gross energy (MJ kg $^{-1}$) calculated according to 23.6 kJ g $^{-1}$ for protein, 39.5 kJ g^{-1} for lipid and 17.0 kJ g^{-1} for NFE

Diet preparation

Mix-Oil including: *T. vulgaris* (%0.5), *O. vulgare* (2%) and *Eucalyptus* spp (2.5%) essential oils in liquid form (Glycole used as carrier) were prepared from Animal Wellness Products Company (Italy). Components of the basal diet (Table 1) were mixed with the obtained Mix-Oil in an appropriate concentration, to get four different experimental diets as follows: With 0% (control group), 50 ppm, 200 ppm and 400 ppm of Mix-Oil. The diets allowed to dry and stored at 4 °C until use. During this study, fish were fed (3 % of body weight) three times per day for 8 weeks.

Gas chromatography mass pectrometry (GC/MS) analysis of Mix-Oil Analyses were performed using a Varian gas chromatograph (Varian Inc., Walnut Creek, California, model HP-6890) equipped with FID and MSD detectors, (Shimadzu, Japan, model 3600) with a DB5 fused silica column (methyl phenyl siloxane, 30 mm length, 0.25 mm i.d.); the carrier gas was helium; split ratio 1:15 and flame ionization detector. The initial temperature of the column was 60 °C (for 2 min) rising to 240 °C at 5 °C min^{-1}, injector temperature 250 ºC and detector temperature 260 ºC. GC-MS analysis was performed on a cross-linked 5% methyl phenyl siloxane used silica capillary column (HP-5, 30m length, 0.25mm id, 0.25µm film thickness). The carrier gas was Helium with a constant flow rate1 ml min^{-1} constant pressure^{-1} 35 Psi, the split ratio 1:15 and temperature program was from 60 ºC (3 min) to 220 ºC at 5 ºC min^{-1}, injector temperature of 260 ºC and detector temperature of 270 ºC. The with a quadrupole mass spectrometer was operated in EI mode at 70 eV ionization energy. Chromatograms were recorded in TIC mode the m/z range was 50 to 500. The retention indices for all the components were calculated by using the retention times of n-alkenes (C8-C25 obtained from Sigma), which were injected after the essential oil under the same conditions (Saharkhiz *et al*., 2012). A total of 36 different components were identified in the Mix-Oil. For Mix-Oil Carvacrol was the predominant compound (45.4%) followed by *Thymol acetate* (10.7%) and *Citraconic anhydride* (2%).

Growth performance

At the end of the feeding trial (8 weeks), the fish were starved for 24 h before weighing and sampling. The following parameters were measured:

Weight gain=W_2(g)-W_1(g)

Specific growth rate (SGR)=100 (LnW2 -LnW1)/T

Feed conversion ratio (FCR)=final weight (g)-initial weight (g)

Average Daily Growth (ADG%)=[Wt_2 –Wt_1/ Wt_1×(t_2–t_1)]×100

Thermal Growth Coefficient (TGC %) = [BW2 $^{0.333}$ –BW1 $^{0.333}$ / $\sum^{0C}$ (day– degrees)] ×100

Daily Growth Coefficient (DGC %)= [BW2 $^{0.333}$ –BW1$^{0.333}$ /(t_2–t_1)]

Condition Factor (CF)=[W/L^3] ×100

Relative Gain Rate (RGR %)=[(W_2–W_1)/W_1]×100

Body weight increase (BWI%)=Final weight-Initial weight/Initial weight× 100 or (Wt_2- Wt_1/Wt_1×100)

Protein Efficiency Ratio (PER)=(g live weight gain /g protein intake)

Lipid Efficiency Ratio (LER)=(g live weight gain/ g lipid intake)

Relative food intake (RFI) = [(g feed eaten /(0.5×(Wt_2-Wt_1)×(t_2-t_1)]×100

W1 is the initial weight, W2 is the final weight and T is the number of days= 56 in the feeding period, t_2 is the first day of the experiment, t_2 is Day 56 of the experiment, L is total length (Tacon, 1990).

In addition, the survival rate (SR, final number of fish/initial number of fish)×100 from each experimental group was evaluated.

Liver enzymes activity

The fish have been fasting 24 h prior to sampling. At first, fish anesthetized with clove oil (100 mg L^{-1}, Sigma Aldrich, Germany) before sample collection (Adel *et al*., 2015). 1 ml blood was drawn from the caudal vein and was transferred to non-heparinized tubes for serum collection (30 fish per group). Serum was collected after centrifugation at 3000 *g* for 15 min, divided into several aliquots and stored at ⁻20 ^{o}C for next study (Saeidiasl *et al*., 2017). Aminotransferase (AST) and alanine aminotransferase (ALT) activities were calculated on fish sera by using commercial kits (Pars Azmoon Company, Tehran, Iran) and a biochemical auto analyzer (Eurolyser, Belgium) (Adel *et al*., 2015).

Histopathological study

At the end of the study, the liver, kidney, spleen and intestine of fish had been removed by dissection of abdominal cavity. The tissue samples were fixed in 10% buffered formaldehyde solution, followed by alcohol dehydration and embedding in paraffin (Sharifpour *et al*., 2014). Six-micrometer thick sections have been stained with haematoxylin-eosin for study of histomorphometrical properties of tissues of rainbow trout and were interpreted under light microscope.

Oxidative stability index (Rencimit test)

3 g of each experimental diets (0% (control group), 50 ppm, 200 ppm and 400 ppm (4 sample per group) of Mix-Oil), crushed, homogenized and weighed into a conical flask. Rencimit test has been measured by Metrohm Rancimat instrument (model 743, Herisau, Switzerland) by method that described by AOCS (1996). That method consists of the creation of substratum extreme stress conditions, with high temperatures (140 °C), and use of oxygen flow (15 L h^{-1}).

Statistical analysis

All the tests were performed triplicate. The data were subjected to statistical analysis using the SPSS software version no. 20 (SPSS Inc., Chicago, IL, USA). After satisfying the assumptions of normality and equal variance, the data have been analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. $p<0.05$ was considered statistically significant.

Results

Fish growth

The effects of Mix-Oil on the growth performance and nutritional parameters of rainbow trout are shown in Table 2 and 3, respectively. At the end of the feeding trial, SGR, ADG, CF, TGC, DGC, RGR and BWI were significantly higher ($p<0.05$) in 200 and 400 ppm Mix-Oil fed groups compared to 50 ppm and control groups. Significantly lower FCR was observed in 200 and 400 ppm Mix-Oil fed groups, with the lowest in 400 ppm group ($p<0.05$). The amounts of PER and LER and the percent's of FCE and RFI was increased as a consequence of dietary administration of Mix-Oil to rainbow trout. Statistically significant increase were observed in PER, LER, FCE and RFI from those fish fed 200 and 400 ppm Mix-Oil enriched diet, respect to the values found on fish from control and 50 ppm groups ($p<0.05$). No significant differences have been observed in the the survival rate percentage between studied groups ($p>0.05$).

Table 2: Growth performance of rainbow trout fed with different levels of Mix-Oil.

Parameter	Control	50 ppm	200 ppm	400 ppm
Initial weight (g)	31.0±0.7[a]	31.2±0.3[a]	30.8±0.5[a]	31.3±0.4[a]
Initial length (cm)	14.1±0.1[a]	13.9±0.1[a]	14.2±0.1[a]	15.0±0.1[a]
Final weight (g)	66.47±0.59[d]	74.93±0.76[c]	83.73±0.61[b]	102.05±0.92[a]
Final length (cm)	16.97±0.1[a]	17.30±0.1[a]	18.90±0.1[a]	19.16±0.1[a]
SGR (%)	1.27±0.02[d]	1.43±0.2[c]	1.74±0.01[b]	1.8±0.02[a]
ADG (%)	1.91±0.02[d]	2.26±0.02[c]	3.06±0.04[b]	3.23±0.05[a]
CF (%)	1.36±0.01[b]	1.45±0.01[a]	1.24±0.01[c]	1.45±0.01[a]
TGC (%)	3.7±0.46[d]	8.7±0.53[c]	17.85±0.36[b]	20.71±0.48[a]
DGC (%)	0.99±0.13[d]	2.32±0.14[c]	4.76±0.1[b]	5.52±0.13[a]
RGR (%)	114.41±1.89[d]	135.64±2.38[c]	183.84±2.07[b]	194.1±2.65[a]
BWI (%)	2482.7±41.02[d]	3019.3±53[c]	3796.3±42.77[b]	4714.7±64.58[a]
SR%	92±1[a]	92±1.5[a]	93±1.4[a]	95±1.2[a]

*Data are presented as mean ± S.D (*n* =15 fish from each group). Means in the same rows with different superscript are significantly different ($p<0.05$)

Table 3: Nutritional parameters of rainbow trout fed with different levels of Mix-Oil.

Parameter	Control	50 ppm	200 ppm	400 ppm
FCR	2.02±0.02[a]	1.88±0.01[b]	1.67±0.01[c]	1.64±0.01[d]
FCE (%)	49.41±0.38[d]	53.3±0.4[c]	59.97±0.24[b]	61.11±0.29[a]
PER	1.22±0.01[d]	1.32±0.01[c]	1.48±0.01[b]	1.51±0.01[a]
LER	2.74±0.02[d]	2.96±0.02[c]	3.33±0.02[b]	3.39±0.02[a]
RFI (%)	6.75±0.05[a]	6.25±0.05[b]	5.56±0.05[c]	5.46±0.03[d]

*Data are presented as mean±S.D (*n*=15 fish from each group). Means in the same rows with different superscript are significantly different ($p<0.05$).

Hepatic function parameters

No significant differences have been observed in the levels of the enzymatic activities of liver (AST and ALT) estimated on fish sera from specimens fed Mix-Oil diets were very similar to the values found for control fish (fed non-supplemented diet) (Table 4). These results suggest that Mix-Oil was not toxic to hepatic health.

Table 4: Hepatic function parameters of rainbow trout fed diets supplemented with different levels of Mix-Oil for 8 weeks.

Parameter	Control	50 ppm	200 ppm	400 ppm
AST (U l^{-1})	323.0 ± 18.4[a]	340.2 ± 20.1[a]	345.5 ± 21.5[a]	336.2 ± 32.3[a]
ALT (U l^{-1})	15.0 ± 0.82[a]	15.0 ± 1.15[a]	16.0 ± 0.82[a]	15.0 ± 0.83[a]

*Data are presented as mean±S.D (*n* =30 fish from each group). Means in the same rows with different superscript are significantly different ($p<0.05$).

Histological results

There was no significant abnormal histological change in the studied tissues in the treatment groups and normal appearance revealed in all groups. In the microscopic examination of the liver and spleen tissue in the treatment groups, no histological changes have been observed.

Normal pattern of renal tubs has observed in kidney of experimental tissues. Microscopic examination of the kidney tissue in the 400-ppm treatment group (Fig. 1), showed an increase in the number of hematopoietic tissue constructors in the kidney and consequently an increase in their density was observed compared to the other studied treatment groups (Fig. 1).

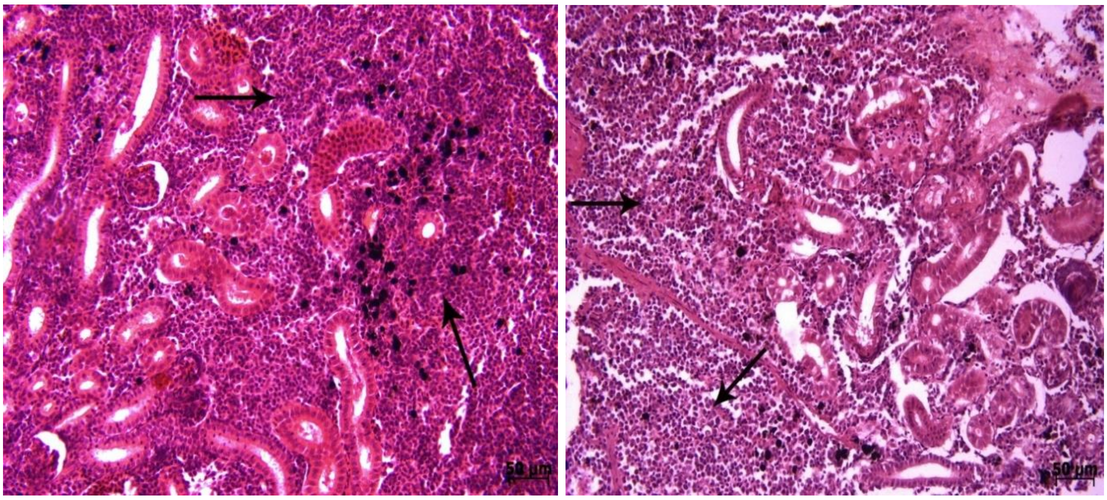

Figure 1: Increase the number of hematopoietic tissue constructors (Arrows) in the kidney of studied treatments. Left picture (400 ppm of Mix-Oil), right picture (Control group).

All studies groups had a same morphology of intestine folds. Statistically significant increase the length of the intestinal villi and a number of epithelium cells in the intestine villi were seen in the 400 ppm treatment group (Fig. 2) compared to the other studied treatment groups (Fig. 2).

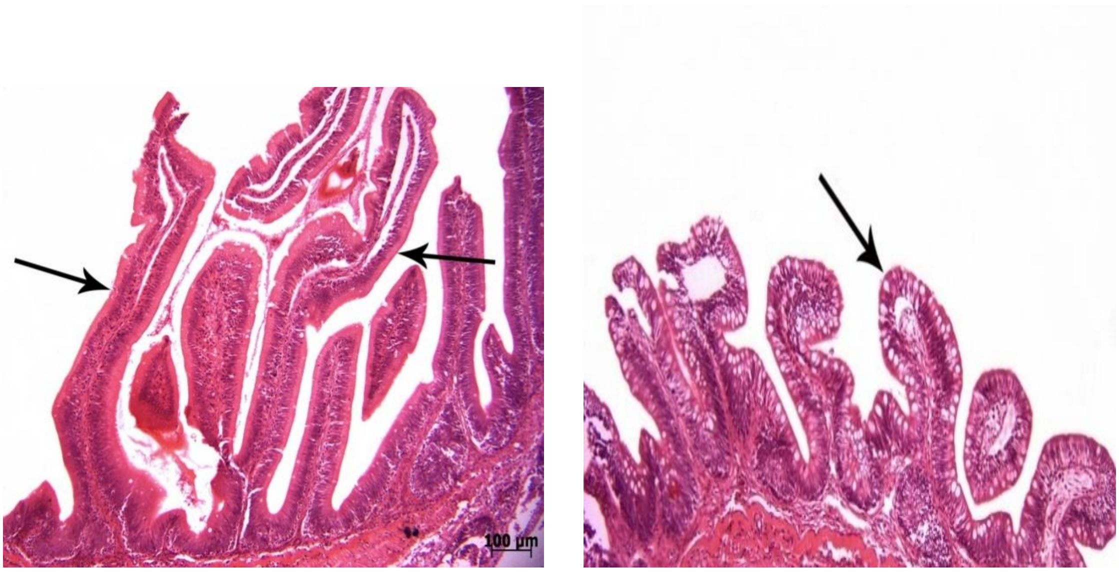

Figure 2: Increase the length of the intestinal villi (Arrows) in the intestine of studied treatments. Left picture (400 ppm of Mix-Oil), right picture (Control group).

Oxidative stability index

Based on the results by increasing the concentration Mix-Oil, trout feed resistance has increased against oxidation and subsequently the shelf life of the feed has increased (Table 5). Although, the observed increments were only statistically significant in the 200 and 400 ppm Mix-Oil (p<0.05) while no significant difference have observed between control and 50 ppm Mix-Oil groups.

Table 5: Results of oxidative stability index of diets containing different levels of Mix-Oil.

Treatment	RANCIMAT^{120} (h)	RANCIMAT^{140} (h)
Control	0.12± 0.01^{c}	0.03± 0.01^{c}
50 ppm	0.12± 0.02^{c}	0.03± 0.01^{c}
200 ppm	0.8± 0.04^{b}	0.2± 0.04^{b}
400 ppm	3.2± 0. 1^{a}	0.8± 0.08^{a}

*Data are presented as mean±S.D (*n*=4sample from each group). Means in the same columns with different superscript are significantly different ($p<0.05$).

Discussion

The herbs and herbal products added to the feed cure many diseases, promote growth, reduce stress, improve immunity and prevent infectious diseases in fish under culture. The addition of herbs and herbal products in fish diet is cheaper and environmental friendly with low side effects to the fish and consumers (Shakya, 2017). The outcomes of this study highlights the growing and immune stimulants effects of some herbal extracts on the rainbow trout through stimulating the immune response and induce protection against disease. The effective roles of three different plant oils have been evaluated on oxidative stability index of trout feed, hepatic function parameters, as well as growth indices that showed significantly higher ($p<0.05$) Mix-Oil fed groups compared to control groups in rainbow trout. Similarly, *M. piperita* (Adel *et al*., 2015), sage and thyme oils (Sönmez *et al*. (2015) and marjoram (*Origanum* spp.) and ajwain (*Trachyspermum ammi*) extracts (Ali *et al.*, 2017) are known to promote growth, feed conversion and/or improve protein digestibility in rainbow trout. Feeding *T. vulgaris*, *O. vulgare* and *Eucalyptus* sp. oil supplemented diets influenced antioxidant improves the liver enzyme activities, growth, FCR and survival rate in varied manners in rainbow trout. Also, AST and ALT ~~is~~ has increased with liver damages and is applied to evaluate liver toxicity and its dysfunction (Sheth *et al.*, 1998). Moreover, serum ALT and AST is generally increased even before the symptoms and clinical signs of the disease onset (Kim *et al*., 2008).

Several investigations have been performed to study the roles of herbal oils on growth performance of fish. However, these studies have been concentrated mainly on health issues. In our study, we evaluate the beneficial roles of the Mix-Oil, such as *M. piperita*, *R. officinalis* and *A. graveolens* oils on rainbow trout growth. At the end of the feeding trial, SGR, ADG, CF, TGC, DGC, RGR and BWI were significantly higher ($p<0.05$) in 200 and 400 ppm Mix-Oil fed groups. In agree with this study, revealed that in a seabass diet with dietary thyme (*T. vulgaris*) could explore the highest energy and protein retentions, as well as slightly improved growth performance at 1% (1000 mgkg-1) thyme powder (Yılmaz, 2012). In another similar study, Sönmez *et al*. (2015), using dietary containing of sage and thyme oils, is effective to improve growth parameters (weight gain percentage and specific growth rate) of rainbow trout after 60 days feeding. On the contrary to our outcomes, it has been reported that oregano, anis and citrus peel essential oils (Hong *et al.*, 2012), and peppermint (Emami *et al.*, 2012) induce better results in higher animals. In this study, SGR values of rainbow trout were positively affected by Mix-Oil treatments. Similar outcomes were reported by Hong *et al.* (2012) and Emami *et al*. (2012) but another study revealed different results from this study on supplementation of sage leaf powder that adversely affected feed conversion rate and growth in broiler (Demir *et al*., 2008).

In this study revealed that Mix-Oil had not toxic to hepatic health, although supplementing at least 100 mg of essential oils blend of *T. vulgaris, M. piperita, R. officinalis* and *Anethum graveolens* could significantly decreased AST and ALT enzyme activities (Mousavi *et al.*, 2017). In accordance with our results, Fernandez *et al*. (1994) reported a remarkable association between dietary consumption of herbal extracts and decreased activities of plasma ALT and AST in broiler chickens with hepatic lesions as well as laying hens under aflatoxin stress. Also, serum ALT and AST activities were decreased in the thyme essential oil-added feed groups of Gibel carp (*Carassius auratus gibelio*) juveniles (Zadmajid and Mohammadi, 2017).

In current study, all studies groups had a same morphology of intestine folds. Statistically significant increase the length of the intestinal villus and the number of epithelium cells in the intestine villus were seen in the 400 ppm Mix-Oil compared to the other studied treatments. In Gurkan *et al.* (2015) survey, improvement in the intestine and liver histology of the *Oreochromismoss ambicus* have been reported after feeding by three spice powders (*T. vulgaris*, *R. officinalis* or *Trigonella foenum graecum*). Also, improvement in proximal intestine and pyloric caeca and increase the epidermal thickness and mucous cell number of rainbow trout received of *Alo evera* 0.1% and 1%were observed (Heidarieh *et al.*, 2013).

After dietary inclusion of herbal oil blend, the normal pattern of renal tubs as well as increase in the number of hematopoietic tissue constructors was observed in kidney. The results showed that the effective interaction between hepatic factors as well as dietary inclusion of herbal oil. On the contrary to our results Mousavi *et al.* (2017) revealed that administration of various sources of dietary fat (mix of *T. vulgaris, M. piperita, R. officinalis and A. graveolens*) had no significant effects on histology of liver. Also, Sönmez *et al.* (2015) showed no histological differences in kidney or liver of rainbow trout fed with 0.5 % and 1 % thyme and sage oil supplemented diets.

Our results also demonstrated that Mix-Oil could increase trout feed resistance against oxidation and subsequently the shelf life of the feed has increased. According with our results several studies also have been reported that herbal essential oils or medical plants have effective role in reducing the optical absorption of free radical because of the activity of hydrogen atom or electron donating and high antioxidant power (Jirovetz *et al.*, 2003; Taheri Gandomani *et al.*, 2014). Therefore, results of our study explore that herbal oil products could have positive effective roles in developed aquaculture, to gain a good growth promotion, feed efficiency, fish health, and elevated stress resistance. Also, it is proposed that these oil plants maybe reveal synergistic effect at the studied dose. The results suggested that dietary administration of Mix-Oil at the level of 400 ppm could improve growth performance of rainbow trout as well as increase the feed stability. Thus, using of Mix-Oil as growth promotor is recommended for farmed rainbow trout. However, further studies on specific mechanisms for immune modulation and diseases resistant should be conducted for exploring the feasibility of application of Mix-Oil in rainbow trout culture.

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References

Adel, M., Amiri, A.A., Zorriehzahra, J., Nematolahi, A. and Esteban, M.Á., 2015. Effects of dietary peppermint (*Mentha piperita*) on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish (*Rutilus frisii kutum*). *Fish and Shellfish Immunology,* 45(**2**), 841-847.

Adel, M., Dadar, M. and Oliveri Conti, G., 2017. Antibiotics and malachite green residues in farmed rainbow trout (*Oncorhynchus mykiss*) from the Iranian markets: A risk assessment. *International Journal of Food Properties,* 20(**2**), 402-408.

Akbarinia, A. and Mirza, M., 2008. Identification of essential oil components of *Thymus daenensis* Celak. In field condition in Qazvin. *The Journal of Qazvin University of Medical Sciences,* 3, 58-62.

Ali, M., Soltanian, S., Akbary, P. and Gholamhosseini. A. 2017. Growth performance and lysozyme activity of rainbow trout fingerlings fed with vitamin E and selenium, marjoram (*Origanum* spp.), and ajwain (*Trachyspermum ammi*) extracts. *Journal of Applied Animal Research*, 46(**1**), 12-23.

AOCS, 1996. Official methods and recommended practices of the American Oil Chemists' Society. 4nd ed. AOCS Press, Champaign. 140 P.

Bachir, R.G. and Benali, M., 2012. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine,* 2(**9**), 739-742.

Chun, S.S., Vattem, D.A., Lin, Y.T. and Shetty, K., 2005. Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. *Process Biochemistry*, 40(**2**), 809-816.

Cross, D.E., McDevitt, R.M., Hillman, K. and Acamovic, T., 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science,* 48(**4**), 496-506.

Dadar, M., Adel, M. and Zorriehzahra, M.J., 2016. Isolation and phylogenic analysis of emerging new antibiotic resistant bacteria, *Acinetobacter lwoffii*, associated with mortality in farmed rainbow trout. *Iranian Journal of Fisheries Sciences*, 15(**4**), 1279-1292.

Demir, E., Kilinc, K., Yildirim, Y., Dincer, F. and Eseceli, H., 2008. Comparative effects of mint, sage, thyme and flavomycin in wheat-based broiler diets. *Archiva Zootechnica,* 11(**3**), 54-63.

De Rosa, S., De Giulio, A. and Iodice, C., 1994. Biological effects of prenyl atedhydroquinones: Structure-activity relationship studies in antimicrobial, brine shrimp, and fish lethality assays. *Journal of Natural Products*, 57(**12**), 1711-1716.

Dorman, H.J.D. and Deans, S.G., 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology,* 88, 308-316.

Emami, N.K., Samie, A., Rahmani, H.R. and Ruiz-Feria, C.A., 2012. The effect of peppermint essential oil and fructo oligosaccharides, as alternatives to *Virginia mycin,* on growth performance, digestibility, gut morphology and immune response of male broilers. *Animal Feed Science and Technology,* 175(**1–2**), 57–64.

Elaissi, A., Salah, K.H., Mabrouk, S., Larbi, K.M., Chemli, R. and Harzallah-Skhiri, F., 2011. Antibacterial activity and chemical composition of 20 Eucalyptus species' essential oils. *Food Chemistry*, 129(**4**), 1427-1434.

Ertas, O.N., Guler, T., Çiftçi, M., DalkIlIç, B. and Simsek, U.G., 2005. The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *International Journal of Poultry Science,* 4(**11**), 879-84.

FAO., 2016. Aquaculture Newsletter. Nutrition-sensitive aquaculture: A timely initiative. Food and Agriculture Organization of the United Nations, Rome. 34P.

Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira, A., Figueiredo, A.C., Barroso, J.G. and Pedro, L.G., 2005. Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L.(Cav.) and *Origanum vulgare* L. *Journal of Agricultural and Food Chemistry,* 53(**21**), 8162-8168.

Fernandez, A., Verde, M.T., Gascon, M., Ramos, J.J., Gomez, J., Lucoand, D.F. and Chavez, G., 1994. Variation of clinical biochemical parameters of laying hens and broiler chicks fed aflatoxin containing feed. *Avian Pathology,* 23, 37–47.

Foysal, M.J., Rahman, M.M. and Alam, M., 2011. Antibiotic sensitivity and in vitro antimicrobial activity of plant extracts to *Pseudomonas fluorescens* isolates collected from diseased fish. *International Journal of Natural Sciences*, 1(**4**), 82-88.

Ghalem, B.R. and Mohamed, B., 2008. Antibacterial activity of leaf essential oils of Eucalyptus globulus and *Eucalyptus camaldulensis*. *African Journal of Pharmacy and Pharmacology*, 2(**10**), 211-215.

Gurkan, M., Yilmaz, S., Kaya, H., Ergun, S. and Alkan, S., 2015. Influence of three spice powders on the survival and histopathology of *Oreochromis mossambicus* before and after *Streptococcus iniae* infection. *Marine Science and Technology Bulletin*, 4(**1**), 1-5.

Heidarieh, M., Mirvaghefi, A.R., Sepahi , A., Sheikhzadeh, N., Shahbazfar, A.A. and Akbari, M., 2013. Effects of dietary Aloe vera on growth performance, skin and gastro intestine morphology in rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Fisheries and Aquatic Sciences*, 13, 367-373.

Hong, J., Steiner, T., Aufy, A. and Lien, T., 2012. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. *Livestock Science*, 144 (**3**), 253–262.

Hoseinifar, S.H., Zou, H.K., Miandare, H.K., Van Doan, H., Romano, N. and Dadar, M., 2017. Enrichment of common carp (*Cyprinus carpio*) diet with medlar (*Mespilus germanica*) leaf extract: Effects on skin mucosal immunity and growth performance. *Fish and Shellfish Immunology*, 67, 346-352.

Jirovetz, L., Buchbauer, G. and Stoyanova, A.S., 2003. Composition, quality control, and antimicrobial activity of the essential oil of long-time stored dill seeds from Bulgaria. *Journal of Agriculture Food Chemistry*, 51, 3854–3857.

Kim, W.R., Flamm, S.L., Di Bisceglie, A.M. and Bodenheimer, H.C., 2008. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology*, 47(**4**), 1363-1370.

Marino, M., Bersani, C. and Comi, G., 1999. Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. *Journal of Food Protection*, 62(**9**), 1017-1023.

Mousavi, A., Mahdavi, A.H., Riasi, A. and Soltani-Ghombavani, M., 2017. Synergetic effects of essential oils mixture improved egg quality traits, oxidative stability and liver health indices in laying hens fed fish oil. *Animal Feed Science and Technology*, 234, 162-172.

Mulyaningsih, S., Sporer, F., Reichling, J. and Wink, M., 2011. Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharmaceutical Biology*, 49(**9**), 893-899.

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A. and Jordán, M.J., 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control,* 19(**7**), 681-687.

Saharkhiz, M.J., Motamedi Kamiar Zomorodian, M., Pakshir, K., Miri, R. and Hemyari, K., 2012. Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. *ISRN Pharmacology,* 13, 127–132.

Saeidiasl, M.R., Adel, M., Caipang, C.M.A. and Dawood, M.A.O., 2017. Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*). *Fish and Shellfish Immunology,* 71, 230-238.

Shakya, S.R., 2017. Effect of herbs and herbal products feed supplements on growth in fishes: A review. *Nepal Journal of Biotechnology,* 5(**1**), 58-63.

Salehi, M., Soltani, M. and Hosseini-Shekarabi, S.P., 2016. Effects of antifungal activity of Daenensis thyme (*Thymus daenensis*) and Mentha (*Mentha longifolia*) essential oils on rainbow trout (*Oncorhynchus mykiss*) eggs hatchability. *Iranian Journal of Aquatic Animal Health,* 2(**2**), 97-107.

Şahin, F., Güllüce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., Agar, G. and Özer, H., 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. *Food control,* 15(**7**), 549-557.

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**), 613-29.

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.

Sharifpour, I., Khoshbavar Rostami, H.A. and Mokarami-Rostami, A., 2014. Sublethal toxicity of organophosphate, diazinon on some organs of great sturgeon (*Huso huso*). *Iranian Journal of Aquatic Animal Health,* 1, 27–35.

Sheth, S.G., Flamm, S.L., Gordon, F.D. and Chopra, S., 1998. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *The American journal of Gastroenterology,* 93(**1**), 44.

Tacon, A.G., 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. Feeding methods. Agent Laboratories Press, Redmond, Taoka. 138 P.

Taheri Gandomani, V., Mahdavi, A.H., Rahmani, H.R., Riasi, A. and Jahanian, E., 2014. Effects of different levels of clove bud (*Syzygium aromaticum*) on performance, intestinal microbial colonization, jejunal morphology and immune competence of laying hens fed different n-6 to n-3 ratios. *Livestock Science*, 167, 236–248.

Yılmaz, S., 2012. Effects of herbal supplements on growth performance of sea bass (*Dicentrarchus labrax*): Change in body composition and some blood parameters. *Energy*, 5, 21-66.

Zadmajid, V. and Mohammadi, C., 2017. Dietary thyme essential oil (*Thymus vulgaris*) changes serum stress markers, enzyme activity, and hematological parameters in gibel carp (*Carassius auratus gibelio*) exposed to silver nanoparticles. *Iranian Journal of Fisheries Sciences*, 16(**3**), 1063-1084.

Zheng, Z.L., Tan, J.Y., Liu, H.Y., Zhou, X.H., Xiang, X. and Wang, K.Y., 2015. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). *Aquaculture*, 292(**3**), 214-218.