Growth performance and lysozyme activity of rainbow trout fingerlings fed with vitamin E and selenium, marjoram (*Origanum* spp.), and ajwain (*Trachyspermum ammi*) extracts

Mohsen Ali\(^a\), Siyavash Soltanian\(^a\), Paria Akbary\(^b\) and Amin Gholamhosseini\(^a\)

\(^a\)Aquatic Animal Health and Diseases Department, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; \(^b\)Department of Marine Sciences, Fisheries Group, Chabahar Maritime University, Chabahar, Iran

**ABSTRACT**

The aim of this study was to investigate the effects of vitamin E and selenium, marjoram (*Origanum* spp.), and ajwain (*Trachyspermum ammi*) extracts on growth performance and lysozyme activity of rainbow trout fingerlings. The results of this study showed that adding ajwain (1%) and marjoram (2%) extracts and Vitamin E plus selenium (1%) significantly increased (*P* < 0.05) the growth factors compared to the control group in rainbow trout. In addition, the survival rate (SR) was 100% after applying the oxygen shock test on trout fed with 1% and 0.5% ajwain extract and 0.5% vitamin E plus Selenium supplement, which was significantly higher than other groups (*P* < 0.05). Another finding showed that 1% Vitamin E plus selenium supplement and diets containing ajwain and marjoram at the level of 1% had significantly the highest value compared to other groups (*P* < 0.05). The measured total antibodies level showed that the groups 1% vitamin E plus Selenium supplement, 1% marjoram, and 1% and 2% ajwain, respectively, had the highest levels compared to the control group. It was shown in the present study that feeding rainbow trout fingerlings with 1% ajwain and marjoram extracts and vitamin E plus selenium as dietary supplements improved growth factor, lysozyme activity and SR.

**1. Introduction**

Lately, the use of natural compounds to strengthen the growth and immunity of aquatic animals is preferred to industrial and chemical compounds. Also, using botanicals as part of the diet ingredients has become popular in aquaculture (Choi et al. 2015; Kakoolaki et al. 2016). On the other hand, one of the negative effects of drugs is to cause resistance in breeding aquatic animals, which has led researchers to evaluate the possibility of replacing the mentioned materials with substances from natural sources, such as medicinal plants as priorities of aquatic animal health research (Akbary et al. 2016). Furthermore, using medicinal plants has several advantages over chemical substances including low cost and no side effects to fish, human health, and environment. Aquaculture is an essential and increasing part in agricultural ecosystems and animal husbandry around the world (Choi et al. 2015; Kakoolaki et al. 2016). Increasing demand for fish, firstly because of rapid population growth, income from this activity and fish precedence over all other animal proteins, as well as cultural and health reasons, has accelerated the developing rate of this industry (Gabor et al. 2010). Food and feeding, essential elements for sustainability and profitability, are crucial in modern aquaculture because the cost of food accounts for about 60–70% of total executive expenses. In addition, it was found that nutrition plays an important role in immune function and disease resistance. As a result, food quality and nutrition management are very sensitive and important (Ebrahimi 2006). In the aquaculture industry, pathogens are one of the product-reducing factors. Today, immune system stimulators are used to solve this problem and since some medicinal plants have a range of useful properties such as boosting and stimulating the immune system, using them can lead to an improvement in aquaculture farms (Choi et al. 2010).

Ajwain belongs to the Apiaceae family. The plant is herbaceous, aromatic, without trichome, with erect stems, 20–50 cm height, and with 6–8 split umbels. The ajwain fruit is small, egg-shaped, with dark yellow colour, and smelling like aroma of thymol, which is consumed in dried and ripened form. Its fruit forms the medicinal organ of this plant (Hedge and Lamond 1987). The major components of the plant extract are thymol, terpinene, phlandrene, pinene group, myrcene, and cymene which are mostly of oxygenated monoterpenes (Nagulakshmi et al. 2000). Its thymol is known as a major chemical compound and as an antibacterial and antifungal drug. Ethanol and acetone in this plant extract are effective against most bacteria such as pseudomonas, *Escherichia coli*, klebsiella, and *Staphylococcus aureus* (Shankaracharya et al. 2000; Usha et al. 2012).

Marjoram is an aromatic plant belonging to the Lamiaceae family and is an important medicinal plant used in most areas around the world. It has a wide distribution in the Mediterranean regions and it grows on rocky slopes with altitudes of 0–4000 m (Afsharypuor et al. 1997). The plant has about
100 cm height and a hairy straight stem which is reddish green in colour. It has oval and dark green leaves covered by trichome on the lower side and the blade is covered with spotted glandular organs. Shoots and leaves of this species are used as an aromatic spice around the world (Alijani et al. 2001). In addition to traditional applications, it is used in the treatment of gastrointestinal diseases, constipation, respiratory disorders (asthma), and as antiseptics. Moreover, the essential oil of this plant is used in pharmaceutical, cosmetic, food, and perfume industries (Kordali et al. 2008). The wild marjoram plant grows in the northern (Chalus, Gilan, and Gorgan), western (Azerbaijan, Ardabil, and Kurdistan) and eastern regions (Ahar and Bojnood) in Iran. Studies have shown many antifungal, antibacterial, and antioxidant activities for essential oils of different species of genus marjoram due to the presence of compounds such as thymol and carvacrol (Muller et al. 1989; Gouladis et al. 2003).

The vitamins as a main group of ingredients in diet are essential for survival and normal growth and breeding of animals. Vitamins act as a catalyst and allow the body to absorb the rest of the food compounds (Han et al. 2011). Vitamin E is a fat-soluble vitamin, which is a major antioxidant in the process of preventing oxidative stress. Vitamin E is composed of two groups of Tocotrienol and Tocopherole substances. Alpha tocopherol is the main form of vitamin E in nature, which is the most stable form among the mentioned derivatives of vitamin E and has the most usage in the animals’ diet (Dandapat et al. 2000; Lu and Liu 2002). Selenium is one of the elements that can play an important role in removing free radicals. In other words, selenium is a kind of indirect form of antioxidant. Recently, this mineral has a key role in animal and livestock nutrition (Rotruck et al. 1973). On the other hand, vitamin E and selenium as a micronutrient combination have synergistic effects on the detoxification of hydroperoxides and protect biological membranes against lipid oxidation (Bell et al. 1985; Combs and Combs 1986). Vitamin E and selenium are mutually interchangeable with each other and each one acts as a resource for the other one. These two vitamins help maintain each other in the cell in different ways. Vitamin E acts against lipid peroxidation and selenium against free radicals (Mirvaghefi et al. 2015). Vitamin E protects fatty acids and selenium protects the thiol group of membranes in red blood cells (Chitra et al. 2013).

Some studies have shown that extracts of various herbs can improve the feed conversion ratio (FCR), decrease the breeding duration, and reduce the breeding cost (Javed et al., 2009). For example, marjoram’s effect on tilapia (Oreochromis niloticus) has led to weight gain (WG) and specific growth rate (SGR), as well as to a reduction in FCR and mortality (Hany, et al. 2014). The addition of thymol as one of the main chemical compositions of ajwain in the diet of rainbow trout in the juvenile phase improves some parameters (Ahmadifar et al. 2011). On the other hand, the presence of two antibacterial substances of thymol and carvacrol inhibiting the growth of various microorganisms has been proven in marjoram and ajwain plants (Abd El-Hack et al. 2016). The combination of vitamin E, selenium to the diet of tilapia (Ahmed et al. 2007), and Atlantic salmon (Salmo salar) (Bell et al. 1987) increased growth rate. On the other hand, the administration of selenium formulas (selenite, selenate, selenomethionine, selenocysteine) in the diet has prevented the mortality of baby salmon (Poston et al. 1976). In addition, the antibody response to changes in the values of Se and vitamin E is sensitive and used as a tool to assess the effects of these two micronutrients on the immune system status (Finch and Turner 1996), so that high Se increases the antibody response in catfish, Lctalurus punctatus (Wang et al. 1997). Deficiency of a combination of vitamin E, selenium caused significant mortality in Channel catfish (Poston et al. 1976; Gatlin et al. 1986).

Rainbow trout (Oncorhynchus mykiss) is one of the most important farmed species in Iran, whose growth and survival always are threatened by a prevalence of infectious and non-infectious diseases, especially during the critical primary stage (Akbary and Jahanbakhshi 2016b; Akbary et al. 2016). The aim of this study is to investigate the effect of two medical plants as well as vitamin E with selenium on growth factors and lysozyme activity of Rainbow trout fingerlings.

2. Materials and methods
2.1. Preparing fish samples, aquariums, and adaptation
In ± 0 g and the mean total length of 2.3 ± 0.1 cm were prepared from one of the province fish farms. Fish were taken in a 200-L tank filled by fish farm water and aerated by an air pump, and then they were transferred to the Laboratory at the Department of Health and Diseases of aquatic animals, School of Veterinary Medicine, Shiraz University. Eleven 70-L aquariums were prepared and then washed and disinfected. One of them was used as a water storage tank, which was aerated and rinsed during the testing (Jahanbakhshi and Hedayati 2013; Akbary and Jahanbakhshi 2016a). A single central air pump was applied for all aquariums with separate branches for each of them and the aeration process was applied throughout the test period; 48 h before transferring new fish into tanks, they were filled with water and completely aerated (Akbary et al. 2016). The adaptation period of fish was considered to be 7 days. During the test period, the mean dissolved oxygen was 8 mg/L, total hardness of carbonate was 250 mg/L, mean temperature was 14.5 ± 1°C, and pH was 7.1 ± 0.5. During the experiment, 10% of the total volume of the tank’s water was replaced daily. After transferring fish to the workshop, there was no feeding until 48 h. After this period, feeding was started with 7–8% of body weight daily; 30 fish per each aquarium were used for categorization after a period of adaptation. Fish were divided and named as following 10 groups, in 3 replications.

2.2. Preparation of plant extracts, vitamin E, selenium, and adding to the diets
First, we completely dried 50 g of ajwain and marjoram plants and then with an electric mill ground them into a white powder; next, aqueous extracts of the plants were obtained using Soxhlet. In the next step, the extracts were completely purified by filter paper to separate the powder deposits from the extract. Then, the extracts were placed in glass plates and were dried in an incubator at a temperature of 35–40°C. Next, the dried extracts were collected from the plates and were...
weighed according to the percentage assigned to the treatment groups (0.5–1–2%) using a digital scale (with 0.001 g accuracy), and then the extracts were dissolved again in distilled water.

Vitamin E with selenium (made in France) was bought in the form of a 1-litre oral solution containing 500 mg of selenium (as sodium selenite) per litre and 100,000 mg of vitamin E per litre that was sprayed at three levels of 0.5%, 1%, and 2% onto the food surface.

The marjoram and ajwain extracts and the vitamin E plus selenium were then added to the basal control diet (Table 1), in appropriate concentrations, to obtain 10 different experimental groups as 0.0 (Control group) 0.5, 1, and 2%. The diets were processed by blending the prepared formulated diets (Table 1) into a homogeneous mixture. Pellets of 1 mm were made by a handmade modified grinder (National, Japan) for which air drying was employed in order to keep moisture approximately at 10% (Table 1), and cooled at 4–8°C until use.

2.3. Oxygen stress test
At the end of the experiment after 30 days, 15 fingerlings were chosen randomly from each treatment group and were taken under the lack of oxygen stress for 6 min. Then, selected larvae from each group were held out of the water and in the air inside the Sachuk net, and after this period were returned back to fresh water to compare the larvae losses and thereby the resistance of fingerlings in the absence of oxygen stress for each treatment group.

2.4. Growth performance factors
Sampling time was determined according to the appropriate time for the effectiveness of the plant extract and the vitamin E plus selenium solution. Sampling was carried out after a month of starting the experiments. In this case, 15 trout fingerlings were taken from each aquarium for biometry so that the total length and weight were measured (Akbar and Jahanbaksh 2016a). Then the fingerlings were transferred immediately to the −70 freezer to assess lysozyme enzyme factors and total antibody level.

Then biometrical calculations were carried out according to the following formula:

- body weight index (BWI) (%): Hung and lutes 1987.

\[ BWI = \frac{(BWF - BWI)}{BWI} \times 10 \]

- Specific growth rate (SGR) (Zhou et al. 2006).

\[ SGR = \frac{(LnWF - LnWi)}{n \times 100} \]

- Feed conversion ratio (FCR) (Zhou et al. 2006).

\[ FCR = \frac{F}{(WF - Wi)} \]

- Condition factor (CF): (Hung and lutes 1987).

\[ CF = \frac{BWF}{(TL3)} \times 100 \]

- Length gain (LG):

\[ LG = \frac{Lf - Li}{10} \]

- Efficient consumer response (ECR):

\[ ECR = \frac{FCR}{*Price} \]

- Survival rate:

\[ Survival Rate = \frac{(initial number - number of losses) \times 100}{100} \]

2.5. Measurements of lysozyme activity level
In order to measure the lysozyme activity in the rainbow trout fingerlings’ tissue, tissue samples were washed several times with normal saline, then larval tissue was weighed and placed in the beaker, and mixed at a ratio of 1 to 10 (W/V) with 1.0 mM sodium phosphate buffer solution which had a pH of 7.1 adjusted with HCl. The homogenization was performed using a homogenizer for 30 s (maximum) for each sample. The mixture of homogenization was poured into 1.5-ml microtube and was transferred to a refrigerated centrifuge (4°C). Serum separation was carried out for 20 min at 10,000 rpm and the supernatant was transferred to a 1-ml microtube and was used to measure the target parameters (Aminlari et al. 2002; Dai et al. 2009; Akbar and Jahanbaksh 2016b).

A spectrophotometer at a wavelength of 450 nm was used for measuring the activity of lysozyme (Harikrishnan et al. 2010). Then 9.2 ml of lysozyme enzyme substrate was prepared in suspension form, including 0.23 mg/ml cell wall of Micrococcus lysodeikticus bacteria within 0.1M sodium phosphate buffer (pH = 7.1) and then 0.1 ml of serum prepared from larvae tissue was added to that. The absorption reduction was recorded at a wavelength of 450 nm over 5 min and the enzymatic activity was calculated based on the slope ratio of the absorption reduction curve within the elapsed time. Each enzyme activity unit defined the amount of enzyme which, at a wavelength of

---

**Table 1.** Formulation and proximate chemical analysis of the basal control diet (% as fed basis).

| Ingredient                     | %      |
|--------------------------------|--------|
| Persian Gulf Lanternfish meal  | 55     |
| Soybean meal                   | 12     |
| Wheat flour                    | 8      |
| Corn meal                      | 5      |
| Fish oil                       | 6      |
| Soybean oil                    | 3      |
| Vitamin premix                 | 4      |
| Mineral premix*                | 2      |
| Binder¹                        | 2.5    |
| Salt                           | 1.5    |
| Anti fungicide                 | 0.5    |
| Antioxidant²                   | 0.5    |
| Proximate composition(%)       |        |
| Crude protein (K)              | 50.1   |
| Crude lipid (%)                | 12.2   |
| Crude ash (%)                  | 12.3   |
| Dry matter (%)                 | 95.37  |
| Moisture (%)                   | 14.2   |
| Fibre (%)                      | 2      |
| Total energy (MJ/kg)           | 21.1   |

*Premix (Harikrishnan et al. 2011).
¹Ametbinder (MehrTaban Yazd, Iran).
²ToxiBan antifungal (Vet-A-Mix, Shenandoah, IA).
³Butylated hydroxytoluene (BHT) (Merck, Germany).
⁴Nitrogen free extracts (NFE)/dry matter (crude protein, crude lipid, ash, fibre).
450 nm and a temperature of 25°C and pH = 7, was associated with a reduction of 0.001 per min in the absorption of the cell wall of *M. lysodeikticus*.

### 2.6. Measurements of total antibody level

The total protein content was measured in serum prepared from tissue using the Biuret/endpoint method and Eppendorf BioPhotometer plus device to determine the total antibody level (Henry et al. 1957). Then the Bromocresol green method was used to measure serum albumin content (Doumas et al. 1971). Finally, the total antibody level was obtained using the difference between measured total protein and albumin.

### 2.7. Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) via SPSS version 16. The mean comparison was calculated using Tukey’s test at a 95% confidence interval and significance level of *P* < .05. Before applying the ANOVA, data normality was examined using the Kolmogorov–Smirnov method.

### 3. Results

#### 3.1. Effect of marjoram and ajwain extracts and vitamin E plus selenium in rainbow trout diet on weight gain index (BWI)

The results of this study after 30 days from starting the experiments showed that fingerlings fed by ajwain and marjoram extracts and vitamin E with Selenium (except to 0.5% E + SEL) caused a significant increase in BWI (*P* < .05) compared to the control group. The results indicated that 1% ajwain caused the highest increase in BWI (65.2 ± 5) compared to other groups, as well as 2% marjoram (53.8 ± 3.5) and 1% vitamin E + selenium (54.5 ± 3.2) caused a significant increase in BWI compared to the control group (27.7 ± 3.5) (Figure 1).

#### 3.2. Effect of marjoram and ajwain extracts and vitamin E plus selenium in rainbow trout diet on SGR

The findings of evaluation of the SGR at the end of the experiments showed a significant increase (*P* < .05) in this indicator in ajwain, marjoram, and E + SEL treatments respectively at the levels of 1% (6.5 ± 0.1); 2% (6.5 ± 0.2); 1% (6.5 ± 0.1) compared to other groups. The control group (5.9 ± 0.1) showed the lowest SGR. Also, 2% ajwain as well as 0.5% and 1% E + SEL treated groups had no significant difference compared to the control group (Figure 2).

#### 3.3. Effect of marjoram and ajwain extracts and vitamin E plus selenium in rainbow trout diet on food conversion ratio

The calculation showed that the highest FCR is related to the control group (1.8 ± 0.19) and the lowest one was related to treatments of 1% ajwain (0.7 ± 0.03); 2% marjoram (0.9 ± 0.04), and 1% E, SEL (0.9 ± 0.05). According to the graph of data analysis, it can be concluded that the addition of plant extracts and vitamin E + SEL at different levels has led to a significant reduction in FCR than the control group (Figure 3).

#### 3.4. Effect of marjoram and ajwain extracts and vitamin E plus selenium in rainbow trout diet on length gain

The results of data analysis show that fingerlings fed with 1% ajwain (2.4 ± 0.4), 2% marjoram (2.1 ± 0.4), and 1% vitamin E + SEL (20.1 ± 0.4) have a significant increase in the LG parameter compared to larvae fed with the basal diet in the control group.
(1.5 ± 0.3). However, other treated groups did not show significant differences compared to the control group (Figure 4).

3.5. Effect of marjoram and ajwain extracts and vitamin E plus selenium in rainbow trout diet on condition factor

The results obtained from CF index or obesity rates showed the highest rate in group E, SEL at the level of 1% (1.04 ± 0.04); furthermore, the treatments of ajwain 1% (0.95 ± 0.01) and marjoram 2% (0.93 ± 0.01) caused a significant increase in this indicator compared to control (0.86 ± 0.02), \(P < .05\) (Figure 5).

3.6. Effect of marjoram, ajwain plant extracts, and vitamin E plus selenium in the diet of rainbow trout on efficient consumer response

The results of analyses showed that the treatments of 1% ajwain (15.8 ± 379.9), 2% marjoram (20.6 ± 459.6), and 1% E + SEL (25.09 ± 453.6) had the lowest ECR level compared with the other groups. In addition, the control group (25.58 ± 900.7) was placed at the highest level of this index compared to the other groups. Generally, the use of ajwain, marjoram herbal extracts, and vitamin E + SEL in the diet of fingerlings reduced the ECR index compared to the control group (Figure 6).
3.7. Effect of marjoram, ajwain extracts, and vitamin E plus selenium in the diet of rainbow trout on SR after the oxygen stress test

The findings of the study showed that the SR of fingerlings fed with 0.5% and 1% ajwain extracts and 0.5% E + SEL was equal to 100% after lack of oxygen stress tests while this index was determined to be about 60% in the control group. The results also showed that adding herbal supplements and vitamins increased the SR of fingerlings compared with the treatments receiving basal diet (Figure 7).

3.8. Effect of marjoram, ajwain extracts, and vitamin E plus selenium in the diet of rainbow trout on lysozyme activity level

Results of measuring the lysozyme activity in the presence of 1% E, SEL supplement showed the highest significant level of 6.46 µg/mg lysozyme compared to other groups. In addition, diets including 1% ajwain and 1% marjoram showed significant lysozyme activity compared to the control group. In addition, no significant difference was observed between 0.5% and 2% E + SEL, 0.5% and 2% marjoram, and 0.5% and 2% ajwain (Figure 8).

3.9. Effect of marjoram, ajwain extracts, and vitamin E plus selenium in the diet of rainbow trout on total antibody level

The results indicated a significant increase in total antibody level in groups of 1% E, SEL, 1% marjoram, 1% ajwain, 2% ajwain (3.5, 2.8, 2.5, and 1.9 g/dL of fingerlings tissue serum). In addition, no significant difference was observed between the 2% E, SEL, and 0.5% ajwain groups. Also 2% marjoram showed no significant level compared to the control group (Figure 9).
4. Discussion

4.1. Effect of adding marjoram and ajwain extracts to the diet on growth factors

The findings of this study showed that the addition of ajwain and marjoram extracts at 1–2% can significantly increase ($P < .05$) some growth factors, including BWI, SGR, FCR, and LG, CF, as well as reduce economic conversion ratio (ECR) in rainbow trout after active feeding. During two studies, the effect of marjoram on growth parameters of tilapia ($O$. nilotica) led to an increase in WG, SGR, and FCR during the experimental period (Hany et al. 2014). Furthermore, the effect of the mentioned plant on growth factors of catfish ($L$. punctatus) also improved WG and FCR (Zheng et al. 2009). The addition of 0.2–0.3 g of thymol as one of the major compositions of ajwain in the diet of rainbow trout in the juvenile phase improves some parameters (Ahmadifar et al. 2011), consistent with the results obtained from this study. The reason for this improvement in growth factors is probably the presence of two important substances, thymol and carvacrol, which can have a favourable effect on the intestinal flora of baby fish owing to antibacterial properties that can provide appropriate potential for growth by positively controlling the intestinal flora and having antimicrobial properties (Ahmadifar et al. 2011). There is also the possibility that thymol and carvacrol affect the body’s metabolic processes and predispose proper digestion and absorption of nutrients, probably due to the favourable effects of these two compounds on the intestinal flora of baby fish. In any case, the effect of these two compounds should be further investigated in future studies.

According to previous studies, the presence of thymol and carvacrol has been proved in marjoram and ajwain plants (Abd El-Hack et al. 2016).

Figure 6. Effect of marjoram, ajwain plant extracts, and vitamin E, selenium in three levels of 0.5%, 1%, and 2% in rainbow trout larval diet on ECR. Different letters represent a significant difference ($P < .05$) among the groups (CONT: control; E, SEL: vitamin E and selenium; marjoram: MARJ; ajwain: AJWA).

Figure 7. Effect of marjoram, ajwain extracts, and vitamin E, selenium at three levels of 0.5%, 1%, and 2% in rainbow trout larval diet on SR. Different letters represent a significant difference ($P < .05$) among the groups (CONT: control; E, SEL: vitamin E and selenium; marjoram: MARJ; ajwain: AJWA).
4.2. Effect of adding vitamin E plus selenium to the diet on growth factors

The data showed that adding 1–2% of vitamin E plus selenium solution significantly increased ($P < .05$) several factors measured, including SGR, weight gain (BWI), FCR, LG, CF, and reduce economic conversion ratio (ECR) in baby fish rainbow trout at the end of the treatment period. However, findings indicated that the level of 1% E + SEL supplement had a better and favourable effect on the growth rate compared to its 2% level. Similar to the results obtained by Bell et al. (1987) on S. salar, vitamin E plus selenium supplement caused significant WG in groups fed the nutrient compared with the control group. Also, feeding trial of selenium supplements in the diet for 30 days had positive effects on the growth of carp (C. gibelio) (Zhou et al. 2009), while the use of supplements of selenium and vitamin E within four weeks had no significant effect on Yellowtail Kingfish (Seriola lalandi). However, after 6 weeks of feeding, Se and vitamin E supplementation showed a significant interaction on growth, as it was found that selenium along with
small amounts of vitamin E increased growth but no increase was observed at higher doses (Le et al. 2014). Our results also revealed that baby rainbow trout fed with 1% E plus selenium supplement than 2% had higher level of growth, which may be due to the same and complementary performance of the two nutrients (selenium and vitamin E) relative to each other. Small amounts of the vitamin can boost small amounts of selenium in enhancing and sustaining growth, but high levels do not have any impact on growth factors. Increased levels of these two compounds beyond a certain amount can show a reverse effect on growth.

According to investigations, there may be interactions between high levels of vitamin E and the selenium element, while low levels of vitamin E together with selenium can enhance the growth rate (Webster and Lim 2002; Le et al. 2014).

4.3. Effect of adding marjoram and ajwain extracts to the diet on increasing SR and level of immune factors (lysozyme and total antibody)

Based on the findings of the present study, the SR was 100% after applying the oxygen shock test on larvae fed with 1% and 0.5% ajwain extract, which was significantly higher than other groups (P < .05). However, all the mentioned supplements showed a greater SR compared to the control group (60%).

In a study, the effects of adding 2% garlic plant to the diets of rainbow trout led to elevated SR (Gabor et al. 2010). The use of parsley (Petroselinum sativum) increased the SR of the common carp (Cyprinus carpio) (Moorkari et al. 2014). The results of all these studies are similar to the findings of the present study, indicating an increase in the SR of baby rainbow trout fed with the marjoram and ajwain extracts.

Probably the reason for this increase in SR is associated with the presence of high amounts of fibre, minerals, vitamins, and thymol in the ajwain. On the other hand, antibacterial, antiparasitic, and antifungal activities of the plant (Zarshenas et al. 2013) can promise great potential to increase SR for baby rainbow trout.

In the present study, the findings of the measurement of serum lysozyme demonstrated that fish fed with diets containing 1% ajwain and marjoram levels showed significantly increased levels of the lysozyme compared with the control group. The findings of a similar study assessing the effect of marjoram extract added to the diets on nonspecific immune responses in 13-g juveniles of rainbow trout showed a significant increase in the activity of serum lysozyme during the second, fourth, sixth, and eighth weeks of sampling compared to the control group (Pourmoghim et al. 2015). The effect of supplemental thyme extract (Boiss Zataria multiflora) on the basal diet of rainbow trout caused a significant increase in the activity of lysozyme (Akbary et al. 2016). On the other hand, oral administration of plant extracts including Astragalus membranaceus, Portulaca oleracea, Flavescent sophora, and A. paniculata in common carp showed inductive effects on immunological parameters, such as increased lysozyme activity (Wu et al. 2007); the results of this study are consistent with the results of the present study. Probably, one of the major reasons for the increased levels of lysozyme in tissue serum of fish larvae fed with the mentioned supplements is the stimulated mucosal cells in different areas of the body including the skin and gastrointestinal tract. The greatest increased levels of total antibodies were found in the groups of 1% marjoram, and 1% and 2% ajwain compared to the control group. During a study, the effect of diet containing thymol and carvacrol was tested on blood parameters in rainbow trout with an average weight of 8 g, so that the number of lymphocytes and macrophages in the fed groups increased significantly (Ahmadifar et al. 2011). Ocimum sanctum leaf extract administered by intraperitoneal injection in Mozambique tilapia fish raised antibody levels in challenge with Aeromonas hydrophila (Logambal et al. 2000).

4.4. Effect of adding vitamin E, selenium to the diet on increasing SR and level of immune factors (lysozyme and total antibody)

According to the results of the SR after applying the oxygen shock test, the baby fish fed with 0.5% of E, SEL supplement had a higher percentage of SR than other groups. Our results were in line with research by Poston et al. (1976) that showed the shortage of nutrients such as vitamin E and selenium separately and simultaneously can significantly reduce mortality in Atlantic salmon fry after four weeks. In a related study, deficiency of a combination of vitamin E plus selenium caused significant mortality in channel catfish (Poston et al. 1976; Gatlin et al. 1986), confirming the results obtained in the present study. Probably, one of the key causes of mortality and decreased SR in juvenile fish can be attributed to an increase in complications arising from a lack of micronutrients such as increased haemolytic rate/decreased haematocrit levels (Watanabe et al. 1997) and decreased growth and reduced resistance in the development of disease. On the other hand, high levels of supplements, particularly elevated amounts of the element selenium, can affect or even reduce the positive effects of supplements because of the interaction with vitamin E.

Data of measuring lysozyme demonstrated that the E + SEL complement (1%) has significantly the highest levels (6.64 μg per milligram) of this enzyme compared to other groups. Similar to our results, activity levels of lysozyme in the Caspian Sea salmon (Salmo trutta caspius) after the addition of vitamins E and C to the basal diet were higher compared to the control group (Sayad Burani et al. 2015). To confirm the findings, the addition of vitamin E and selenium to the diet of Yellowtail Kingfish (S. islandi) improved the level of lysozyme. The results of this study showed that the lysozyme activity in the absence of vitamin E and the presence of selenium alone as a supplement in the diet could not affect the levels of lysozyme. On the other hand, high levels of vitamin E together with selenium increase the levels of lysozyme. The above-mentioned findings confirm the need for the simultaneous use of these two micronutrients in the diet of the species (Le et al. 2014). In a related study, the addition of 200 mg per kilogram of vitamin E to the diet of Nile tilapia juveniles in a challenge with Streptococcus iniae led to increased activity of lysozyme.
One reason cited is the increase in the leukocyte count and phagocytic activity (Lim et al. 2009).

Increased levels of lysozyme can be attributed to the presence of both supplements in the diet of baby fish, possibly through the synergistic effect on the immune system, elevated number of white blood cells producing lysozyme, and stimulate the mucosal cells in tissues responsible for digestion such as the intestine.

In examining the total antibody level as well as other biochemical parameters measured, Group E + SEL 1% showed the highest increase compared to the control group. The results of other similar studies demonstrated that the use of vitamin E in the diet of *Chanos chanos* (Forsskal) (Azad et al. 2007) and supplementation of selenium to the diet of channel catfish (*L. punctatus*) (Wang et al. 1997) raised the level of antibody response. In addition, the lack of vitamin E in the diet of trout reduced antibody levels and suppressed immune responses (Blazer and Wolke 1984). Le et al. (2014) found that the separate and simultaneous uses of these micro-nutrients in the diet of Yellowtail Kingfish (*S. lalandi*) had no effect on the amount of antibodies in this species. The reason for the difference in the antibody response to these supplements may be related to differences in species, ecological conditions, different nutritional needs, and different ways of stimulating the immune system.

These factors are indicators for the growth and nutritional performance in early life and are important because most deaths occur in early life. Our goal of adding these supplements is their effect on the growth performance and immunity of early life.

5. Conclusion

Given that the cost of food accounts for the main bulk of investment in aquaculture, it seems necessary to further research this issue in order to achieve appropriate, inexpensive, and safe nutritional supplements to enhance the efficiency of fish growth and SR.

In the present study, the improvement of growth factors was evaluated as effective following the prescription of ajwain and marjoram herbal extracts and vitamin E plus selenium at 1% as dietary supplements in the diet of rainbow trout fingerlings. In general, the benefits of dietary supplements include increasing production, reducing the length of the growth period, reducing losses, increasing resistance to pathogens, reducing water pollution, and improving the physicochemical farming environment.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the Aquatic Animal Health and Diseases Department, School of Veterinary Medicine at Shiraz University. Also, this study was conducted by using credits at the Shiraz University.

References

Abd El-Hack ME, Alagawany M, Ragab Farag M, Tiwari R, Karthik K, Dhama K, Zorriezhzahra J, Adel M. 2016. Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. J Essential Oil Res. 28:1–14.

Afsharypour S, Sajjadi ES, Erfan-Manesh M. 1997. Volatile constituents of *Origanum vulgare* ssp. viride (syn. *O. heracleoticum*) from Iran. Planta Med. 63:179–180.

Ahmadifar E, Falahatkar B, Akrami R. 2011. Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout. *Onchorhynchus mykiss*. J Appl Ichthyol. 27:1057–1060.

Ahmed K, El-Hammyadi I, Ibrahim SA, El Kasheif MA. 2007. Synergistic reactions between vitamin E and selenium in diets of hybrid tilapia (*Oreochromis niloticus* *Oreochromis aureus*) and their effect on the growth and liver histological structure. Egypt J Aquat Biol Fish. 11:53–81.

Akbary P, Gharaghanipoor M, Fereiduni MS. 2016. The effect of thyme (*Boiss Zataria multiflora*) and pennyroyal (*Mentha pulegium*) L on changes in phagocytosis, lysozyme, respiratory burst and blood cells in rainbow trout (*Onchorhynchus mykiss*). J Vet Res. 70:65–72.

Akbary P, Jahanbakhshi A. 2016a. Effect of starvation on growth, biochemical, hematological and non-specific immune parameters in two different size groups of grey mullet, * Mugil cephalus* (Linnaeus, 1758). Acta Ecologica Sinica. 36:205–211.

Akbary P, Jahanbakhshi A. 2016b. Growth yield, survival, carcass quality, haematological, biochemical parameters and innate immune responses in the grey mullet (*Mugil cephalus*) fingerling induced by immunogen* pre-biotic. J Appl Anim Res. 1:1–7.

Akbary P, Pirbeigi A, Jahanbakhshi A. 2016. Analysis of primary and secondary stress responses in bighead carp (*Hypophthalmichthys nobilis*) by anesthetization with 2-phenoxethanol. Int J Environ Sci Technol. 13:1009–1016.

Alijani N, Kapolutzakis E, Mitaku S, Chinou IB. 2001. Composition and antimicrobial activity of the essential oils of two *Origanum* species. J Agricult Food Chem. 49:4168–4170.

Aminlari M, Li A, Kunanithy V, Scaman CH. 2002. *Rhodanese distribution* in porcine (*Sus scrofa*) tissues. Comp Biochem Physiol Part B. 132:309–313.

Azad IS, Dayal JS, Poornima M, Ali SA. 2007. Supra dietary levels of vitamins C and E enhance antibody production and immune memory in juvenile milkfish, *Chanos chanos* (Forsskal) to formalin-killed *Vibrio vulnificus*. Fish Shellfish Immunol. 23:154–163.

Bell JG, Cowey CB, Adron JW, Hie BJS. 1987. Some effects of selenium deficiency on enzyme activities and indices of tissue peroxidation in Atlantic salmon parr (*Salmo salar*). Aquaculture. 65:43–54.

Bell JG, Cowey CB, Adron JW, Shankis AM. 1985. Some effect of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (*Salmo gairdneri*). Br J Nutr. 53:149–157.

Blazer VS, Wolke RE. 1984. The effects of a-tocopherol on the immune response and non-specific resistance factors of rainbow trout (*Salmo gairdneri* Richardson). Aquaculture. 37:1–91.

Chitra P, Edwin SC, Moorthy M. 2013. Effect of dietary vitamin E and selenium supplementation on Japanese quail broilers. Dep Poultry Sci Vet College Res Inst, Namakkal. 43:195–205.

Choi IH, Park WY, Kim YJ. 2010. Effects of dietary garlic powder and (alpha)-tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken. Poult Sci. 89:1724–1731.

Choi YH, Lee BJ, Nam TJ. 2015. Effect of dietary inclusion of *Pyropia yezoensis* extract on biochemical and immune responses of olive flounder *Paralichthys olivaceus*. Aquaculture. 435:347–353.

Combs GF, Combs SB. 1986. Biochemical functions of selenium. In: The role of selenium in nutrition. Toronto: Academic Press; p. 206–265.

Dai W, Fu L, Du H, Jin C, Xu Z. 2009. Changes in growth performance, metabolic enzyme activities, and content of Fe, Cu, and Zn in Liver and Kidney of Tilapia (*Oreochromis niloticus*) exposed to dietary Pb. Biol Trace Element Res. 128:176–183.

Dandapat J, Chainy GB, Rao KJ. 2000. Dietary vitamin-E modulates antioxidant defence system in giant freshwater prawn, *Macrobrachium rosenbergii*. Comp Biochem Physiol. 127:101–115.
Doumas BT, Watson WA, Biggs HG. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chem Acta. 31:87–96.

Ebrahimi I. 2006. Nutrition and needs of fish in aquaculture. Isfahan: Jahad Esfahan University Press.

Finch JM, Turner RJ. 1996. Effects of selenium and vitamin E on the immune responses of domestic animals. Res Vet Sci. 60:97–106.

Gabor EF, Sara A, Barbu A. 2010. The effects of some Phyto-additives on growth, health and meat quality on different species of fish. Anim Sci Biotecnol. 43:61–65.

Gatlin DM, Poe WE, Wilson RP. 1986. Effects of singular and combined dietary deficiencies of selenium and vitamin E on fingerling channel catfish (Ictalurus punctatus). J Nutr. 116:1061–1067.

Gouladis M, Tzakoy O, Verykokidoy E, Harvala C. 2003. Screening of some Greek aromatic plants for antioxidant activity. Phytother Res. 17:194–195.

Han D, Xie S, Liu M, Xiao X, Liu H, Zhu X, Yang Y. 2011. The effects of dietary selenium on growth performances, oxidative stress and tissue selenium concentration of gibel carp (Carassius auratus gibelio). Aquac Nutr. 17: e741–e749.

Hany MR, Abdel-Latif R, Khalil H. 2014. Evaluation of two Phytobiotics, Spirulina platensis and Origanum vulgare extract on growth, Serum anti-oxidant activities and resistance of Nile tilapia (Oreochromis niloticus) to pathogenic Vibrio alginolyticus. Int J Fish Aquat Stud. 1:250–255.

Harikrishnan R, Balasundaram C, Heo MS. 2013. Lactobacillus sakei BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, Epinephelus bruneus. Fish Shellfish Immun. 29:1037–1043.

Harikrishnan R, Balasundaram C, Heo MS. 2011. Influence of diet enriched with green tea on innate humoral and cellular immune response of kelp grouper (Epinephelus bruneus) to Vibrio carchariaeinfection. Fish Shellfish Immun. 30:972–979.

Hedge IC, Lamond JM. 1987. Trachyspermum. Flora Iranica. 162:336

Hung SSO, Iutes PB. 1987. Optimum feeding rate of hatchery produced crude oil on serum biochemical changes in the great sturgeon Acipenser transmontanus at 20°C. Aquaculture. 65:307–317.

Jahanbakshi A, Hedayati A. 2013. The effect of water-soluble fraction of crude oil on serum biochemical changes in the great sturgeon Huso huso. Comp Clin Pathol. 22:1099–1102.

Javed M, Durrani FR, Hafeez A, Khan RU, Ahmad I. 2009. Effect of aqueous extract of plant mixture on carcass quality of broiler chicks. ARPN J Agric and Bio Sc. 4:37–40.

Kakoolaki S, Akbary P, Zorriehzahra MJ, Salehi H, Sepahdari A, Afsharnasab Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E. 2008. Antifungal, Lim C, Yildirim-Aksoy M, Menghe HL, Thomas LW, Phillip HK. 2009. Influence of dietary levels of lipid and vitamin E on growth and resistance of Nile tilapia to Streptococcus iniae challenge. Aquaculture. 298:76–82.

Logambal SM, Venkatalkalshmi S, Michael RD. 2000. Immunostimulatory effect of leaf extract of Ocimum sanctum Linn.in Oreochromis mossambicus (Peters). Hydrobiologia. 430:113–120.

Lu C, Liu Y. 2002. Interaction of lipoic acid radical cations with vitamins C and E analogue and hydroxycinnamic acid derivatives. Arch Biochem Biophys. 406:78–84.

Mirvaghefi A, Ali M, Asadi F. 2015. Effects of vitamin E, selenium and VITAMIN C on Various biomarkers following oxidative stress caused by Diazinon exposure in Rainbow Trout. J Aquac Mar Biol. 2:1–9.

Mooraki N, Dadgar SH, Naderi MS. 2014. The effect of parsley (Petroselinum sativum) on the growth and survival of koi fish (Cyprinus carpio). J Aquatcul Develop. 8:63–72.

Muller J, Reisinger G, Muhlbauer W. 1989. Drying of medicinal and aromatic plants in a greenhouse solar dryer. Landtechnik. 2:58–65.

Nagolakshmi S, Shankaracharya NB, Naik JP, Rao LJM. 2000. Studies on chemical and technological aspects of ajowan aspects (Trachyspermum ammi). J Food Sci Technol Mysore. 39:277–281.

Poston HA, Combs GF, Leibovitz L. 1976. Vitamin E and selenium inter-relations in the diet of Atlantic salmon (Salmo salar): gross, histological and biochemical signs. J Nutr. 106:892–904.

Pourmohghim H, Haghighi M, Sharif Rohani M. 2015. Effect of Dietary inclusion of Origanum vulgare extract on nonspecific immune responses and Hematological parameters of Rainbow trout (Oncorhynchus mykiss). Bull Environ Pharmacol Life Sci. 4:33–39.

Rottruk JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science. 179:588–590.

Sayad Burani M, Khara H, Sayad Burani M, Fakharzadeh SE. 2015. The effect of different levels of vitamin C and vitamin E supplement in the diet on growth performance and immune system of the Caspian Sea salmon. Iran J Fisheries Sci. 23:95–96.

Shankaracharya NB, Nagolakshmi S, Naik JP, Rao L. 2000. Studies on chemical and technological aspects of Ajowan (Trachyspermum umammi) syn (Carum coticum) seeds. J Food Sci Techn. 37:277–281.

Usma M, Ragini SH, Naqvi SM. 2012. Antibacterial activity acetone and ethanol extracts of Cinnamon (Cinnamomum zeylanicum) and Ajowan (Trachyspermum ammi) on four food spoilage bacteria. Iran Res J Biol Sci. 1:7–11.

Wang C, Lovell RT, Klesius PH. 1997. Response to Edwardsiella icteriui challenge by channel catfish fed organic and inorganic sources of selenium. J Aquat Anim Health. 9:172–179.

Watanabe T, Kiron V, Satoh S. 1997. Trace minerals in fish nutrition. Aquaculture. 151:185–207.

Webster CD, Lim CE. 2002. Introduction to fish nutrition. In: Webster, CD, Lim, CE editors. Nutrient requirements and feeding of fish for aquaculture. Oxon: CABI Publishing; p. 1–27.

Wu G, Yuan C, Shen M, Tang J, Gong Y, Li D, Huang C, Han X. 2007. Immunological and biochemical parameters in carp (Cyprinus carpio) after Qompsell feed ingredients for long-term administration. Aquaculture. 151:255–266.

Zheng ZL, Tan JYW, Liu HY, Zhou XH, Xiang X, Wang KY. 2009. Evaluation of oregano essential oil (Origanum heracleoticum L.) on growth, antioxidant effect and resistance against Aeromonas hydrophila in channel catfish (Ictalurus punctatus). Aquaculture. 292:214–218.

Zhou X, Wang Y, Gu Q, Li W. 2009. Optimal dietary methionine requirement for juvenile cobia (Rachycentron canadum). Aquaculture. 258:551–557.

Zhou X, Wang Y, Gu Q, Li W. 2009. Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase activity of crucian carp (Carassius auratus gibelio). Aquaculture. 291: 78–81.