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PROOF

Effects of Dietary Supplementation of Herbal Oils Containing 1,8-cineole, Carvacrol or Pulegone on Growth Performance, Survival, Fatty Acid Composition, and Liver and Kidney Histology of Rainbow Trout (*Oncorhynchus mykiss*) Fingerlings

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Abstract

A feeding trial was performed to assess any beneficial effect of three herbal oils; mint, thyme and sage oils containing 74 g kg-1 pulegone, 77 g kg-1 carvacrol and 76 g kg-11,8-cineole, respectively on growth performance, survival, fatty acid composition, and liver and kidney histology of rainbow trout (*Oncorhynchus mykiss*). Herbal oils were added at 0.5 %, 1.0% and 1.5 % concentrations to a fish meal-based control diet, and each diet was fed to triplicate groups of rainbow trout fingerlings for 60 days with growth parameters recorded weekly. Results showed that FCR was affected negatively in all mint oil diet fed groups where it was higher than that of control and other groups (P<0.05). The lowest FCR values were recorded in the thyme and sage oil containing diets fed groups (P<0.05) with no significant differences between these two groups. SGR was also found lower in mint oil groups than in control. The highest SGR was recorded in thyme and sage oil fed groups (P<0.05). Survival was similar in fish fed with diets containing thyme, sage oil and control diet (P>0.05). However, survival was the lowest in fish fed with mint oil supplemented diets(P<0.05). Whole body fatty acid profiles except total saturated fatty acids are closely influenced by dietary herbal oil administrations. Total polyunsaturated n-6 fatty acid was lowest in 1 %thyme oil and all sage oil diet fed groups (P<0.05). Total polyunsaturated n-3 fatty acid was similar between control and sage oil supplemented groups, but in other groups it was significantly decreased (P<0.05). EPA/DHA ratio was observed significantly low in 1.5 %thyme oil and all groups of sage oil fed fish. There were no histological differences observed in liver or kidney of fish fedwith control and 0.5 % and 1 % thyme and sage oil supplemented diets.The present results showed positive effects of thyme and sage herbal essential oil supplemented diets on growth performance and fatty acid utilization and these oils may be useful feed supplements in production of rainbow trout.

Keywords: Rainbow trout, feed additive, herbal oil, growth performance, survival, fatty acid composition.

1,8 Kinol, Karvakrol ya da Pulegon Içeren Bitkisel Yağ Yem Katkılarının Alabalık Yavrularında (Oncorhynchus Mykiss) Büyüme Performansı, Yaşama, Yağ Asidi Kompozisyonu ile Karaciğer ve Böbrek Histolojisi Üzerine Etkileri

Özet

Sırasıyla 74 g kg-1 pulegon, 77 g kg-1 karvakrol ve 76 g kg-1 1,8-kinol içeren nane, kekik ve adaçayı gibi üç farklı bitkisel yağın alabalıkların (Oncorhynchus mykiss) büyüme performansı, yaşama, yağ asidi kompozisyonu ile karaciğer ve böbrek histolojisi üzerine etkilerini belirlemek amacı ile bu besleme çalışması yürütülmüştür. Bitkisel yağlar balık yemi içerisine % 0,5, % 1 ve % 1,5 oranında eklenmiş, ve her diyet büyüme parametreleri haftalık olarak kontrol edilen 60 günlük çalışmada üç tekerrürlü olarak alabalık yavrularına verilmiştir. FCR tüm nane gruplarında olumsuz etkilenirken diğer tüm gruplardan yüksek tespit edilmiştir (P<0,05). En düşük FCR oranları kekik ve adaçayı içeren gruplarda belirlenmiştir (P<0,05) ve bu gruplar arasında farklılık tespit edilememiştir. SGR nane grubunda kontrol grubunda olduğundan daha düşük çıkmıştır. En yüksek SGR değerleri kekik be adaçayı içeren gruplarla beslenen alabalıklarda gözlenmiştir (P<0,05). Yaşama gücü, kekik, adaçayı ve kontrol grubunda benzer olmuştur (P>0,05). Bununla birlikte nane yağı ile beslenen gruplarda düşük tespit edilmiştir (P<0,05). Toplam doymuş yağ asidi hariç tüm yağ asitleri içeriği bitkisel yağ eklentisi ile değişiklik göstermiştir. Toplam çoklu doymamış n-6 yağ asitleri % 1 kekik ve tüm adaçayı içeren gruplarda en düşük değeri göstermiştir (P<0,05). Toplam çoklu doymamış n-3 yağ asitleri kontrol ve adaçayı yağı ekleniş gruplarda benzerken, diğer gruplarda önemli derecede düşmüştür (P<0,05). EPA/DHA oranı % 1,5 kekik yağı ve tüm adaçayı yağı içeren gruplarda kayda değer düşüktür. Kontrol, % 0,5 ve % 1 kekik ve adaçayı yağı içeren gruplarda karaciğer ya da böbrek histolojilerinde bir farklılık gözlenmemiştir. Bu çalışma sonuçları kekik ve adaçayı ile desteklenmiş yemlerin büyüme performansı ve yağ asidi kullanımı üzerindeki olumlu etki gösterdiğini ortaya koymuştur ve bu yağlar alabalık üretiminde yem katkısı olarak kullanışlı olabileceği belirlenmiştir.

Anahtar Kelimeler: Gökkuşağı alabalığı, yem katkısı, Bitkisel yağ, büyüme performansı, yaşama, yağ aside kompozisyonu.

Introduction

Major tasks in aquaculture industry are to maintain fish health as well as to improve fish performance. Fish producers use a large quantity of antibiotics and chemicals to prevent and control diseases (Harikrishnan *et al*., 2011). However, the use of antibiotics in fish farming is restricted in many countries due to increasing development of antibiotic resistance in aquatic bacteria (Citarasu, 2010). Also, the occurrence of antimicrobial residues in aquaculture products threatens human health (WMO, 2006).

Nowadays, medicinal herbs are becoming more popular than ever before as far as the possible adverse effects of synthetic drugs are concerned (Rawling *et al*., 2009; Bilen *et al*., 2011; Bilen *et al*., 2013; Bilen *et al* 2014). Therefore, there are increasing concerns about testing potential impacts of natural additives on health monitoring and growth effects in fish (Citarasu, 2010).

The herbal oils have been in use for different purposes, such as food, drugs and perfumery since ancient times (Heath, 1981). They are also considered among the most important antimicrobial agents present in the plants, and may also have antioxidant and antiinflammatory activities (Nonato *et al*., 2011; Biswas *et al*., 2012). Many of them were evaluated for poultry, swine and cattle production, while their potential in fish diets has yet to be established. Therefore, the aim of present study is to investigate the effects of supplementation of essential oils of sage, thyme and mint on the growth performance, survival, fatty acid composition, and liver and kidney histology of rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Fish and Experimental Condition

Rainbow trout, average body weight of 13.3 ± 0.08 g, were provided from Research Center of Faculty of Fisheries at Atatürk University. Fish were kept in 1 m^3 tanks supplied with freshwater flow-through arrangement for 15 days prior to the experiment. The experiment had 9 dietary treatments with 3 replicates divided into 30 aquariums (45-L) containing 10 fish in each. During the study, water quality was temperature 17±0.1°C, pH 7.4±0.2, dissolved oxygen 8.10±0.2 mg/L and total NH_3 0.00±0.01 mg/L.

Essential oil of mint (*Mentha spicata*), thyme (*Thymus vulgaris*) and sage (*Salvia* sp.) were purchased from NURİ AGA LTD and added to a commercial trout extruded feed (Pınar™, Turkey) at a dose of 0.5, 1.0and 1.5 % by a mixer. The diets were prepared according to the previously described protocol (Sönmez *et al*., 2015).Mint, thyme and sage oils contained 74 g kg^{-1} pulegone, 77 g kg^{-1} carvacrol and 76 g kg^{-1}1,8-cineole, respectively. Control diet contained no herbal oil. Diets with different doses of sage, thyme and mint were designated as M 0.5 %, M 1.0 %, M1.5 %, T 0.5 %, T1.0 %, T1.5 %, S 0.5 %, S1.0 % and S1.5%,, respectively. Fish were fed with the diets three times in everyday as *ad libitum* for 60 days.

Proximate and Fatty Acid Composition

Moisture, crude protein, lipid and ash of the experimental diets and fish carcass were determined by following methods of AOAC (2000). Fatty acid compositions of the fish carcass were determined according to Miller *et al*. (2006).

Histological Examination

Changes on liver and kidney histology of rainbow trout were examined on the last day of the experiment. Five fish from each tank were euthanized by overdose of MS-222 (200 $mgL^{-1}$of water for 10 min) for organs collection. Organs were removed carefully, fixed immediately in a 10% buffered formalin, dehydrated in a graded ethanol series, and embedded in paraffin. Sections were cut at 5 µm, and stained with hematoxylin and eosin for light microscopic examination (Hisar *et al*., 2002). Organ sections were examined for general abnormalities, such as inflammation, loss or regeneration of tissue, and nuclear location.

Growth and Survival Performance

Growth parameters and survival were calculated as per the methods described previously (Wojno, 1977; Laird and Needham 1987; Fowler 1991)

Data Analysis

Before performing statistical analysis, the data were subjected to normality checking and they followed normal distribution. One-way analysis of variance (ANOVA) was performed followed by Tukey-Kramer HSD for multiple comparisons of means. The level of significance was considered at 5%. All statistical analyses were performed using SPSS for Windows v. 17.0 program (SPSS Inc., Chicago, IL, USA).

Results

At the end of the trial, a significant difference (P<0.05) was found in final weight of rainbow trout fingerlings fed with different test diets (Table 1). FCR was increased in all mint-diet groups and found higher than in control and other groups (*P*<0.05) (Table 1). Significantly lower FCR values were recorded in the fingerlings fed with thyme and sage oil containing diets (P<0.05) with no differences between these latter two treatments. Similarly, SGR

Table1. Effects of three levels of mint, thyme and sage oil supplemented diets on growth, feed conversion and survival in rainbow trout fingerlings

Diet	Final weight (g)	FCR	SGR (% d^{-1})	Survival (%)
Control	56±0.25[b]	0.91±0.09[a]	2.42±0.08[b]	100 [a]
M 0.5 %	45.4±0.19[a]	1.28±0.07 [b]	2.14±0.05[a]	84±2.5[b]
M 1.0 %	46.7±0.23[a]	1.21±0.17 [b]	2.12±0.07[a]	65±3[c]
M1.5 %	43±0.25[b]	1.36±0.11 [b]	1.99±0.06[a]	65±3[c]
T 0.5 %	63.2±0.35 [c]	0.81±0.19[c]	2.61±0.12 [c]	100 [a]
T 1.0 %	63±0.24 [c]	0.81±0.11 [c]	2.58±0.13 [c]	100 [a]
T 1.5 %	64.5±0.47 [c]	0.79±0.15[c]	2.61±0.06 [c]	100 [a]
S 0.5 %	69.3±0.47 [c]	0.73±0.01[c]	2.77±0.06 [c]	100 [a]
S 1.0 %	68.2±0.51[c]	0.75±0.5[c]	2.76±0.13 [c]	100 [a]
S 1.5 %	68.1±0.34 [c]	0.75±0.16[c]	2.75±0.08 [c]	100 [a]

Diets with three inclusion levels (500, 1000 and 1500 mg kg^{-1}) of mint, thyme and sage oils are denoted as M 0.5 %, M 1.0 %, M 1.5 %, T 0.5 %, T 1.0 %, T 1.5 %, S 0.5 %, S 1.0 % and S 1.5 %, respectively. All data represent the mean ± S.E.M. Within a column, values with different superscripts are significantly different from each other (P<0.05). SGR, specific growth rate; FCR, feed conversion ratio.

was also noticed to be decreased in groups fed with mint oil included diets comparing to control groups (Table 1). SGR was higher in thyme and sage oil treated groups compared to control and groups fed with mint oil containing diets (P<0.05).

Thyme and sage oil supplemented diets fed fish had similar survival with control (P>0.05) (Table 1). However, mint essential oil supplemented diets caused significantly low survival (P<0.05).

In the study, no differences were noticed on the total saturated fatty acid (SFA) composition of rainbow trout fed with essential herbal oil supplemented diets (Table 2). Total monounsaturated fatty acid (MUFA) was determined to be the highest in thyme oil fed groups (P<0.05). Eicosoneic acid (20:1n-9) was higher in mint and thyme oil treated fish groups than in control and sage groups (P<0.05) with lowest value in control. Similar results were observed on oleic acid (18:1n-9) levels, but no significant differences were observed between control and sage oil fed groups. Total polyunsaturated n-3 fatty acid value was similar between control and sage oil fed fish groups (P>0.05), but the values were different in thyme and mint oil treated from those control and sage oil fed fish groups. Eicosapentaenoic acid (EPA, 20:5n-3) was found to be higher in all groups than in control (P<0.05). However, the highest value of EPA was observed in 1.0 %mint oil fed group. Docosahexaenoic acid (DHA, 22:5n-3) level was higher in control and sage oil containing diet fed groups with no significance. Total polyunsaturated n-6 fatty acid level was the lowest in T 1.0 % and all sage oil fed groups (P<0.05). Total polyunsaturated n-3 fatty acid content was similar with control and sage oil fed groups, but it was comparatively lower in other treatment (P<0.05). Level of arachidonic acid (20:4n-6) was the highest in T 1 % treatment. EPA/DHA ratio was observed significantly low in 1500 $mgkg^{-1}$ thyme and all sage oil supplemented diet administered fish groups (P<0.05).

Histological analysis of liver and kidney in control fish did not indicate any pathological changes. However, vascular congestion (Figure 1a) and focal tubule necrosis (Figure1b) were observed in liver and kidney from fish of M 1.0 % and M 1.5 % groups, respectively. Hydropic degeneration occurred in liver of rainbow trout from T 1.5 % and S 1.5 % groups (Figure1c). However, there were no histopathological differences in the liver or the kidneys of control, M 0.5 %,0.5 % and 1.0 % thyme and sage oil fed groups.

Discussion

To date, several studies were conducted to evaluate effects of medical herbs on growth of fish. However, these investigations have focused mainly on health effects. In this study, we tried to take account of beneficial effects of the some herbal essential oils, such as mint, thyme and sage oils on rainbow trout.

There are reports on positive growth effects of sage, mint and thyme on broiler and animals (Demir *et al*., 2008; Emami *et al*., 2012; Hong *et al*., 2012;),but effective doses for trout were unknown. We chose to begin with 0.5 %, 1.0 % and 1.5 % doses that had good impact. Especially, all doses of sage and thyme caused better weight gain, SGR and FCR. Some recent studies have exhibited that feeding fish with thyme resulted in an elevated disease resistance and improved survival, which may be attributed to an improvement of immune functions (Ergün *et al*., 2011; Yılmaz *et al*., 2013). Yılmaz *et al*. (2012b) also evaluated the use of thyme (*T. vulgaris)* in a diet for seabass and they demonstrated that 1% (1000 $mgkg^{-1}$) thyme powder produced the highest protein and energy retentions, and slightly improved growth performance. In the study, feeding mint essential oil supplemented diets to rainbow trout resulted in low SGR and high FCR values. On the contrary to our results, peppermint (Emami *et al*., 2012), and oregano, anis and citrus peel essential oils (Hong *et al*., 2012) provided better results in higher animals.

SGR and FCR values of rainbow trout were positively affected by thyme and sage oil treatments. Similar results were observed by Emami *et al*., 2012 and Hong *et al*., 2012, but Demir *et al*. (2008) had

Table 2. Carcass fatty acid composition of the rainbow trout fed three levels of mint, thyme and sage oil supplemented diets

Fatty acid	Control	M 0.5 %	M 1.0 %	M 1.5 %	T 0.5 %	T 1.0 %	T 1.5 %	S 0.5 %	S 1.0 %	S 1.5 %
14: 0	1.25±0.02^{b}	3.82±0.07^{a}	3.56±0.03^{a}	4.14±0.29^{a}	3.96±0.03^{a}	4.19±0.06^{a}	3.95±0.06^{a}	1.32±0.08^{b}	1.33±0.10^{b}	1.33±0.03^{b}
15: 0	-	0.48±0.01	0.44±0.01	0.62±0.53	0.52±0.03	0.25±0.25	0.48±0.02	0.34±0.04	-	-
16: 0	18.08±0.15^{b}	13.85±0.09^{a}	13.33±0.34^{a}	15.28±0.55^{a}	13.17±0.07^{a}	14.60±0.96^{a}	13.58±1.17^{c}	17.87±0.19^{b}	17.32±0.27^{b}	17.35±0.74^{b}
17: 0	0.29±0.03^{c}	0.48±0.05^{a}	0.48±0.02^{a}	0.75±0.04^{b}	0.49±0.04^{a}	0.59±0.10^{a}	0.52±0.02^{a}	0.40±0.03^{a}	0.45±0.01^{a}	0.42±0.01^{a}
18: 0	4.79±0.40^{b}	3.23±0.08^{a}	3.06±0.08^{a}	3.13±0.28^{a}	2.88±0.12^{a}	3.05±0.07^{a}	2.77±0.41^{a}	4.35±0.14^{b}	4.53±0.03^{b}	4.54±0.12^{b}
20: 0	-	1.06±0.05^{a}	1.09±0.08^{a}	0.24±0.03^{b}	1.50±0.40^{a}	0.56±0.01^{c}	0.79±0.01^{d}	-	-	-
Σ SFA	24.40±0.56	22.93±0.14	21.96±0.56	24.15±0.01	22.52±0.45	23.24±0.94	22.10±1.55	24.27±0.24	23.63±0.40	23.64±0.84
14:1	0.20±0.02^{a}	0.18±0.01^{a}	0.17±0.01^{a}	0.52±0.01^{b}	0.17±0.02^{a}	0.51±0.01^{b}	0.20±0.01^{a}	0.31±0.01^{c}	0.36±0.03^{c}	0.34±0.01^{c}
15:1	1.32±0.22^{a}	0.08±0.00^{b}	0.08±0.00^{b}	0.16±0.03^{b}	0.67±0.01^{c}	0.12±0.04^{b}	0.68±0.00^{c}	2.21±0.15^{d}	1.50±0.08^{a}	1.37±0.01^{a}
16:1n-7	1.10±0.01^{a}	5.13±0.10^{b}	5.16±0.07^{b}	5.33±0.38^{b}	5.24±0.02^{b}	5.53±0.14^{b}	5.66±0.36^{b}	1.52±0.08^{a}	1.54±0.06^{a}	1.70±0.11^{a}
17:1	0.19±0.01^{a}	0.12±0.01^{a}	0.34±0.01^{a}	0.39±0.07^{ab}	0.55±0.05^{b}	0.29±0.01^{a}	0.56±0.02^{b}	0.95±0.01^{c}	0.73±0.04^{d}	0.74±0.01^{d}
18:1n-7	1.75±0.08^{a}	3.04±0.07^{b}	2.94±0.04^{b}	2.89±0.08^{b}	2.98±0.12^{b}	2.98±0.09^{b}	3.11±0.07^{c}	2.65±0.10^{b}	2.22±0.08^{d}	2.27±0.05^{d}
18:1n-9	8.95±0.33^{a}	15.32±0.17^{b}	14.81±0.17^{b}	15.16±0.26^{b}	15.47±0.99^{b}	16.28±0.78^{a}	16.82±0.14^{c}	9.10±0.01^{a}	8.26±0.30^{a}	8.20±0.53^{a}
20:1n-9	0.86±0.04^{a}	1.52±0.09^{b}	1.38±0.15^{b}	1.20±0.08^{b}	1.35±0.09^{b}	1.26±0.06^{b}	1.42±0.01^{b}	0.87±0.07^{c}	0.58±0.01^{d}	0.61±0.08^{d}
22:1n-9	0.43±0.07^{a}	0.40±0.05^{a}	0.54±0.04^{a}	0.50±0.03^{a}	1.05±0.27^{b}	0.65±0.11^{ab}	0.72±0.03^{c}	0.72±0.06^{c}	0.70±0.03^{c}	0.65±0.01^{ab}
24:1n-9	1.23±0.01^{a}	0.41±0.02^{b}	0.49±0.12^{b}	0.38±0.06^{b}	0.63±0.15^{c}	0.28±0.09^{d}	0.54±0.04^{b}	0.70±0.17^{c}	0.58±0.15^{c}	0.86±0.36^{c}
Σ MUFA	16.02±0.15^{a}	26.20±0.08^{b}	25.90±0.50^{b}	26.51±0.08^{bc}	28.12±1.09^{c}	27.89±1.04^{c}	29.70±0.48^{c}	19.03±0.05^{a}	16.45±0.46^{a}	16.73±1.12^{a}
18:3n3	1.66±0.24^{a}	1.24±0.04^{b}	1.21±0.03^{b}	1.24±0.06^{b}	1.24±0.06^{b}	1.19±0.01^{b}	1.22±0.06^{b}	0.76±0.05^{c}	0.54±0.06^{d}	0.55±0.06^{d}
18:4n-3	0.30±0.06^{a}	1.17±0.01^{b}	1.24±0.02^{b}	1.08±0.16^{c}	1.05±0.01^{c}	1.06±0.06^{c}	1.13±0.11^{c}	1.04±0.05^{c}	0.70±0.04^{d}	0.78±0.10^{d}
20:4n-3	0.84±0.01^{a}	0.76±0.01^{a}	0.81±0.03^{a}	0.75±0.10^{a}	0.89±0.08^{a}	0.85±0.10^{a}	0.90±0.01^{a}	0.26±0.26^{c}	0.34±0.34^{c}	0.26±0.26^{c}
20:5n-3	6.32±0.15^{a}	7.72±0.12^{b}	8.39±0.19^{c}	7.10±0.41^{b}	7.06±0.20^{b}	7.08±0.08^{b}	7.65±0.79^{b}	6.05±0.03^{a}	7.04±0.10^{b}	7.40±0.21^{b}
22:5n-3	2.30±0.01^{a}	3.01±0.24^{c}	2.86±0.24^{c}	2.36±0.19^{a}	3.07±0.74^{c}	2.32±0.00^{a}	2.36±0.15^{a}	2.00±0.29^{a}	1.54±0.17^{b}	2.20±0.06^{b}
22:6n-3	41.09±1.27^{a}	27.23±0.34^{b}	28.42±1.27^{b}	28.58±0.82^{b}	26.86±0.18^{b}	28.11±0.85^{b}	25.40±0.46^{b}	40.57±0.91^{a}	43.77±1.34^{a}	42.53±0.79^{a}
Σ PUFA n-3	52.50±1.30^{a}	41.13±0.49^{b}	42.93±1.78^{b}	41.10±0.09^{b}	40.18±0.37^{b}	40.61±0.77^{b}	38.65±1.57^{b}	50.68±1.06^{a}	53.93±0.95^{a}	53.72±0.42^{a}
18:2n-6	7.26±0.06^{a}	6.99±0.16^{a}	7.06±0.12^{a}	7.00±0.19^{a}	6.78±0.27^{a}	7.36±0.35^{a}	2.84±0.33^{b}	2.62±0.25^{b}	2.60±0.27^{b}	5.37±0.54^{c}
20:4n-6	1.86±0.30^{a}	1.62±0.31^{a}	0.45±0.01^{b}	1.34±0.32^{c}	1.02±0.55^{c}	2.00±0.19^{d}	0.41±0.04^{b}	0.30±0.09^{b}	0.39±0.21^{b}	0.65±0.03^{bc}
22:2n-6	0.31±0.11^{a}	0.35±0.10^{a}	0.09±0.09^{b}	0.34±0.13^{a}	0.13±0.01^{c}	0.46±0.09^{d}	-	-	-	-
Σ PUFA n-6	9.42±0.24^{a}	8.97±0.24^{a}	7.60±0.02^{b}	8.67±0.38^{a}	7.94±0.81^{b}	9.82±0.63^{a}	3.25±0.29^{c}	2.92±0.16^{c}	2.99±0.06^{c}	6.02±0.57^{d}
Σ n-3/n-6	4.37±0.16^{a}	4.80±0.33^{a}	5.41±0.01^{a}	4.64±0.24^{a}	5.18±0.62^{a}	3.94±0.09^{a}	15.77±1.73^{b}	18.53±0.70^{c}	18.00±0.49^{c}	8.18±1.24^{d}
EPA/DHA	0.28±0.01^{a}	0.30±0.01^{a}	0.25±0.02^{a}	0.26±0.01^{a}	0.25±0.01^{a}	0.30±0.03^{a}	0.15±0.01^{b}	0.16±0.01^{b}	0.17±0.01^{b}	0.15±0.01^{b}

Diets with three inclusion levels (500, 1000 and 1500 mg kg^{-1}) of mint, thyme and sage oils are denoted as M0.5 %, M1.0 %, M1.5 %, T 0.5 %, T 1.0 %, T1.5 %, S 0.5 %, S 1.0 % and S1.5 %, respectively. All data represent the mean ± S.E.M. Within a row, values with different superscripts are significantly different from each other ($P<0.05$). SFA; Saturated Fatty Acids. MUFA; monounsaturated fatty acids. PUFA; polyunsaturated fatty acids; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

different results from our study on sage leaf powder supplementation that negatively affected growth and feed conversion in broiler. In our study, survival was negatively affected by mint oil supplemented diet in rainbow trout.

EPA/ DHA ratio is the most important index for optimum fish development (Skalli and Robin, 2004). The fish were growing, and it was expected that fatty acids would be oxidized extensively to provide energy for growth. In our study, EPA/DHA did not show differences among treatment groups except all sage and T1.5 % groups. Similar result was observed on n-3/n-6 ratio and PUFA n-6. In addition, in fish groups of T 0.5 % and T 1 %, PUFA n-3 decreased. These results exhibited that sage and thyme oil fed fish groups had increased fatty acid utilization which might have improved fish growth. Furthermore, some fatty acid levels were increased in herb oil supplemented fish groups and some of them were decreased. Therefore, it is suggested that herbal oil supplementations may affect conversion of fatty acids in a varied fashion. Dietary EPA and DHA are considered as essential fatty acids in marine fish. However, some studies demonstrated that freshwater fish, such as rainbow trout, ayu, eel, and tilapia can convert linolenic acid to EPA and DHA, even though the ratio of conversion was different among the species (Takeuchi, 1997).

Histopathological changes may provide insight into the effects of unhealthy diets on various tissues and organs in fish. Therefore, in present study, the histological analyses were performed to assess any signs of damage in liver and kidney associated with dietary incorporation of mint, thyme or sage oils. In the present study, liver and kidney histopathology of the control fish showed a normal histological architect without indication of any deformity. However, abnormal histological findings, i.e. congestion, necrosis or degeneration were observed in liver and kidney samples of fish fed with high level of herbal oil containing diets.

Negative effects of herbal oils on growth performance, survival, fatty acid composition or histological changes may be related to toxic constituents, excessive doses, or allergic conditions, but they generally have no effects on health when used in the proper doses and application (Bandaranayake, 2006; Yılmaz *et al*., 2012a). The results of the present study demonstrate that inappropriate herbal oil levels exerted negative effects on growth, survival, fatty acid composition and organ histology of fish. Our findings were similar to the previous studies with *Oreochromis mossambicus* and *O. niloticus* fed with cumin powder (Yılmaz *et al*., 2012a) and caraway seed meal (Ahmad and Abdel-Tawwab 2011), respectively.

The present results displayed positive effects of sage and thyme herbal essential oil supplemented diets on growth performance and fatty acid utilization in rainbow trout. Further work is needed to explore

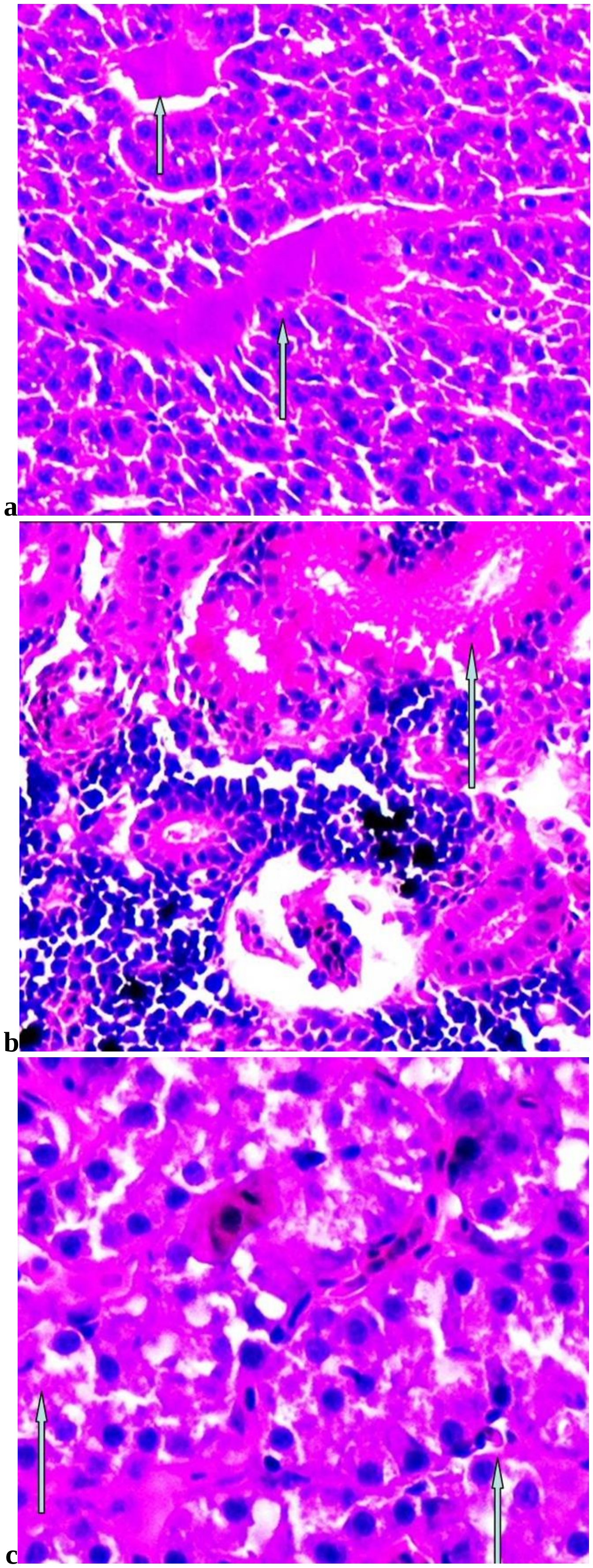

Figure 1. Liver (a and c) and kidney (b) histology from rainbow troutfed high level of herbal oil containing diets.a: Vascular congestion in mint and sage, b: focal tubule necrosismint and sage and c: hydropic degeneration in mint.

impact of sage and thyme essential oils on fish immunity and disease resistance.

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