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# EFFECT OF DIETARY SUPPLEMENTATION OF *Melissa Officinalis* and *Aloe Vera* ON HEMATOLOGICAL TRAITS, LIPID OXIDATION OF CARCASS AND PERFORMANCE IN RAINBOW TROUT (*Oncorhynchus Mykiss*)

**A. FARAHI[1]*, M. KASIRI[1], M. SUDAGAR[1], M. SOLEIMANI IRAEI[2], S.M.J. ZORRIEHZAHRA[3]**

*[1]Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*
*[2]Department of Marine Sciences and Technologies, Hormozgan University, Bandarabbas, Iran*
*[3]Iran Fisheries Research Organization*

*Email: farahi2010@yahoo.com

**ABSTRACT:** *This was conducted investigate the effect of feeding lemon balm (Melissa officinalis) and Aloe (Aloe vera) on growth performance, hematological parameters and oxidative stability of rainbow trout. 360 uniform rainbow trout (20.87±0.25 g) were divided into 3 groups, and fed standard diets supplemented with ground lemon balm (2%, L group) or supplemented with Aloe (1%, A group) and without supplementation (Control, C group). Growth performance and body composition were not influenced by plant supplementation. Survival rate of fish was promoted in diets supplemented with herbs, significantly (P<0.05). A significant enhancement (higher value) of WBC and Hct was found in supplementation compared with control (P<0.05). However, any significant differences (P>0.05) were not observed in RBC and Hb in treatments (P>0.05). Results of thiobarbituric acid value (TBA) showed that lemon balm and Aloe herbs could be protective against lipid peroxidation in fish meat during chilling storage (4 °C, 7 days).*

**Key words:** *Lemon balm (Melissa officinalis), Aloe (Aloe vera), plants supplementation, Rainbow trout (Oncorhynchus mykiss)*

ORIGINAL ARTICLE

## INTRODUCTION

The increasing pressure on the aquaculture to reduce or eliminate feed antibiotics as growth enhancers has initiated new research to find safe and efficient natural alternatives. This new generation of feed additives includes herbs and their essential oils and extracts (Brenes and Roura, 2010). Herbal additives contain substances which increase also appetite and digestion (Barreto et al., 2008). There has been published many studies have confirmed that the addition of plants or their extracts in the diets has a beneficial effect to improve growth parameters and protect from diseases in aquaculture. (Shalaby, 2004; Sasmal et al., 2005; Johnson and Banerji, 2007, Farahi et al. 2010, Sudagar et al., 2010; Kasiri et al., 2011).

Medicinal plants are the main sources of natural antioxidants. Generally, fish fat contains higher levels of polyunsaturated fatty acids (PUFA). This is due to the relatively high content of phospholipids in the membrane structure of the muscle cells (Bystricky and Dicakova, 1998). Just a higher degree of unsaturation of fatty acids in muscle membranes is related to increasing of their susceptibility to oxidation of meat and meat products (Marcincak et al., 2010). Lipid oxidation is one of the primary mechanisms of quality deterioration in food, especially in meat products (Gorelik et al., 2008). Therefore, there is a need to increase the antioxidant capacity of muscles, what can be achieved by feeding of antioxidant active substances. Using of natural antioxidants in fish diets is a simple method to achieve higher antioxidant stability, improve sensory properties and prolongate the storage of fish. Important source of natural antioxidants is plant material; lemon balm is common among herbs with high proportion of antioxidant active substances (Marcincak et al., 2008a).

The effect of feeding of lemon balm (*Melissa officinalis*) and Aloe (*Aloe vera*) on growth performance of rainbow trout, body composition, fatty acid profile, and oxidative stability of produced fish was investigated.



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## MATERIALS AND METHODS

### Experimental design and fish

The study was carried out at Ghezelkohpayeh fish farm, Haraz, Iran. 360 uniform rainbow trout (20.87±0.25 g) were purchased from a commercial hatchery. Fishes were randomly divided into 3 groups (n=120) in 3 replications, each containing 40 fish. The rainbow trout were transferred to the place of experiment and acclimated for 2 weeks. During the acclimation, fish were fed the experimental diet to satiation twice a day at 09:00 and 15:00. After acclimation, fish were fasted for one day, batch weighted and randomly distributed among nine troughs. Water temperature was 14-16°C, $O_2$ 7-8 $mgl^{-1}$, pH 7-8 and light: dark cycle of 12: 12 h was maintained during the feeding trial.

The dietary treatments consisted of the same diets. The ingredients and chemical composition of the diets are shown in Table 1. Experimental group (L group) was fed using diet with addition of ground lemon balm in dose 20 g per 1 kg of diet. Experimental group (A group) was fed using diet with addition of Aloe in dose 10 g per 1 kg of diet. Control group (C group) was fed basal diet without any supplementation. During the experiment, fish were fed the experimental diet to satiation third a day at 08:00, 12:00 and 16:00.

**Table 1 - Formulation and proximate composition of the basal diets**

| Ingredients | (%) |
|---|---|
| Fish meal | 50 |
| Wheat meal | 20 |
| Soybean meal | 12 |
| Fish oil | 10 |
| Vitamin premix [a] | 1.5 |
| Mineral premix [b] | 1.5 |
| Filler | 5 |
| *Proximate composition* | |
| Crude protein | 40.48 |
| Crude lipid | 17.60 |
| Ash | 11.26 |
| Wet | 1.48 |
| Energy (kJ) | 3.83 |

[a] Vitamin A, 3600000 IU; Vitamin D3, 800000 IU; Vitamin E, 14.4 g; Vitamin K3, 0.8 g; VitaminB1, 0.71 g; Vitamin B2, 2.64 g; Vitamin B6, 1.176 g; Vitamin B9, 0.4 g; Niacine, 11.88; Ca D-pantothenate, 3.92 g; Choline chloride, 100 g; Vitamin B12, 6 mg; H2, 4mg. [b] Mn, 39.68 g; Zn, 33.88 g; Fe, 20 g; Cu, 4 g; I, 397 mg; Se, 80 mg; Choline chloride, 100 g.

### Chemical composition

Proximate composition of diets and tissues were specified by using the Association of Analytical Chemists (AOAC, 2003) methods. Dry matter was determined by oven drying at 550°C. The crude protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method. Lipids were isolated in ground samples (fish carcass) with petroleum ether with Soxhlet apparatus and were determined gravimetrically (Folch et al., 1957).

### Hematological Analysis

The indices used to evaluate the hematological profile were included; white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb) and hematocrite (Hct). The procedures were based on methods described for fish hematology (Houston, 1990).

### Evaluation of thiobarbituric acid assay

To determine the lipid oxidation changes of thigh meat, the method of thiobarbituric acid value (TBA) determination, expressing the degree of secondary damage of lipids, contingent upon the oxidation of unsaturated fatty acids, was used. Examination of samples was carried out on 1, 4, and 7 days of storage at chilling conditions (4°C). The extent of lipid oxidation was evaluated as thiobarbituric acid reactive substances (TBARS) by the method of Marcinčák et al. (2004). TBARS values were measured spectrophotometrically at 532 nm (Helios γ, v. 4.6, Thermo spectronic, Cambridge, UK). Results were quantified as malondialdehyde (MDA) equivalents (mg $MDA.kg^{-1}$ muscle).

### Calculations and statistical analysis

The following variables were calculated: Body weight increase (BWI) = Wt – W0; Specific growth rate (SGR) = (ln Wt – ln W0) ×100 $t^{-1}$; Feed conversion ratio (FCR) = total dry feed consumed (g) / total wet weight gained (g); HSI : [wet liver wt. (g) ×100] / wet body wt. (g); Wt and W0 were final and initial fish weights (g), respectively; and t is the experimental period in days.

All the data were analyzed statistically using SPSS Software, Version 16.00. One-way analysis of variance (ANOVA) with the post hoc Duncan's multiple comparison tests was used to evaluate statistical significance of differences among the control and experimental groups. The results are given as means, standard error of the mean (SEM) and $P<0.05$ was considered as statistically significant difference.



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## RESULTS

### Fish growth performance

The effect of lemon balm (*Melissa officinalis*) and Aloe (*Aloe vera*) supplementation on body weight, feed conversation ratio (FCR), specific growth rate (SGR) and hepatosomatic index (HSI) are presented in Table 2. No differences (P>0.05) were observed among treatments in growth parameters.

**Table 2 - Performance of rainbow trout (mean ± SD) in experimental groups**

| Parameters | Control | Group L | Group A |
|---|---|---|---|
| BWI | 82.97±4.39 | 80.70±7.30 | 84.06±3.18 |
| FCR | 1.17±0.05 | 1.23±0.06 | 1.13±0.01 |
| SGR | 1.22±0.06 | 1.15±0.2 | 1.28±0.04 |
| HIS | 2.37±0.44 | 2.33±0.35 | 2.32±0.41 |

Values in the same row are not significantly different (P > 0.05). Group L= ground lemon balm, Group A= Aloe

### Survival rate

As far as Figure 1 is concerned, remarkable difference (P<0.05) in fish survival was observed, as significant promotion was obtained in plants supplementation trails.

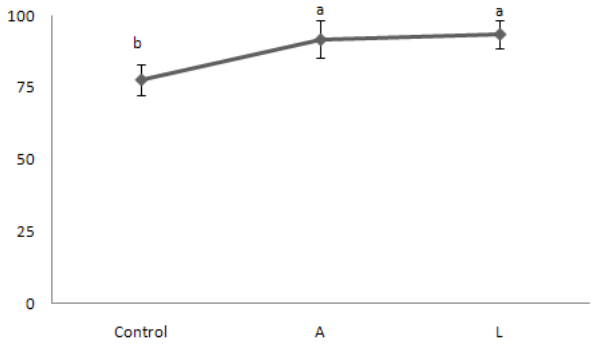


Figure 1 - Survival rate (Mean ± SD) in experimental groups

### Chemical composition

As can be seen in the Table 3, results showed that plants supplementation had no influence on the carcass (P>0.05).

### Hematological parameters

A significant enhancement of WBC and Hct was found in supplementation compared with control (P<0.05). Inversely, any significant differences (P>0.05) were observed in RBC and Hb in treatments.

**Table 3 - Body composition of fish in experimental groups**

| Parameters | Control | Group L | Group A |
|---|---|---|---|
| Crude protein | 17.44±0.22 | 17.83±0.87 | 17.47±0.25 |
| Crude lipid | 6.20±1.30 | 5.79±0.30 | 5.50±0.18 |
| Ash | 3.75±0.25 | 4.10±0.45 | 3.93±0.41 |
| Wet | 72.45±1.10 | 72.28±0.18 | 72.1±1.25 |

Values in the same row are not significantly different (P > 0.05). Group L= ground lemon balm, Group A= Aloe

### Lipid oxidation of stored meat

Table 4 shows results of the determination of TBA value measured in fish muscles stored in refrigerator (4°C, 1, 4 and 7 days). On the first day of samples storage were levels of TBA products (TBARS), expressed as the amount of MDA, generally low in all groups. However, already on the first day of storage, the amount of TBARS in both experimental groups (A, L) was lower compared to control (P<0.05). Following storage of samples, caused that TBARS values in all groups were gradually increased (P<0.05), but the amount of TBARS in the control group was significantly higher in comparison with experimental groups (P<0.05). The lowest levels of TBARS and thus the lowest fat damage throughout the storage period were recorded in samples A.

**Table 4 - Hematological parameters of fish in experimental groups**

| | | | |
|---|---|---|---|
| WBC (×10³/ $\mu$L) | 19.00±5.20$^{b}$ | 41.66±10.43$^{a}$ | 38.45±7.25$^{a}$ |
| RBC (×10⁶/ $\mu$L) | 0.69±0.26 | 0.82±0.30 | 0.72±0.27 |
| Hb (g/dL) | 9.37±1.82 | 8.04±0.35 | 7.17±0.08 |
| Hct (%) | 26.34±5.04$^{b}$ | 52.47±8.25$^{a}$ | 49.25±5.16$^{a}$ |
| WBC (×10³/ $\mu$L) | 19.00±5.20$^{b}$ | 41.66±10.43$^{a}$ | 38.45±7.25$^{a}$ |

Values in the same row are not significantly different (P > 0.05). Group L= ground lemon balm, Group A= Aloe

## DISCUSSION

The effect of medicinal herbs and their essential oils or extracts on the growth performance of fish has been described by Salah et al. (2008), Farahi et al. (2010) and Kasiri et al. (2011). Though these studies have stated the promotion of growth parameters by usage of plants supplementation diets, our study revealed that there are not any differences in growth parameters in experimental groups. The resulting growth promoting effect of plants or plant extracts used as feed additives depends on their proper concentrations, composition of basal diet and management and husbandry conditions (Barreto et al., 2008, Nasir and Grashorn, 2010). Our study was carried out at ideal experimental conditions, which could affect the degree of growth promotion.



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Nasir and Grashorn (2010) indicate that feeding *Nigella sativa* and a combination *Nigella sativa* and *Echinacea purpurea* extract did not affect carcass of broilers. Similarly, our findings implied that no remarkable effect of plants supplementation diets was found on body composition of fish. However, Farahi et al. (2010) reported the significant effect of garlic addition to the diets on body composition of rainbow trout.

Many studies showed that application of medicinal plants supplementation diets cause hematological parameters to improve, clearly (Salah et al., 2008; Sudagar et al., 2010; Farahi et al., 2011). Our results revealed that amount of WBC and Hct were enhanced in the groups A and L. As a result, we can assert that immunity of fish was increased in these group compared with control. Having had better hematological characteristics and immunity, survival rate in groups A and L was promoted, obviously. As far as fish survival is concerned, mortality at the end of experiment was declined by medicinal herbs supplementation diets. This result is in agreement with Salah et al. (2008) and Kasiri et al. (2011).

Lipid oxidation in meat and meat products (apart from microbial spoilage) is the primary process by which quality loss occurs. Recently, scientific research has been focused on the use of antioxidant properties of natural plants and their extracts in animal nutrition due to the stabilization of fat in produced meat (Marcincak et al., 2008b; Lahucky et al., 2010; Luna et al., 2010). The results of the present study indicate that lemon balm and Aloe had a positive effect on the oxidative stability of fish meat during chilling storage. The lower lipid oxidation of experimental groups could be related to the antioxidant characteristics of plants. Lemon balm and Aloe are rich in phenol compounds that exhibit antioxidant properties (Sokol- Letowska et al., 2006; Marcincak et al., 2008a). Studies have shown that phenol compounds (flavonoids, proanthocyanidins) have the capacity to act powerful antioxidant activity by scavenging free radicals and terminating oxidative reactions (Sayago-Aerdi et al., 2009). Florou-Paneri et al. (2006) investigated the effect of feeding oregano and oregano extract on oxidative stability of turkey meat. They also noted that the administration of 10 $g.kg^{-1}$ ratio of oregano or 200 $mg.kg^{-1}$ of oregano extract reduced fat oxidation, while at higher dosages oxidation is lower.

## CONCLUSION

To sum up, considering the fact that application of *Melissa officinalis* and *Aloe vera* was not efficient in growth performance of rainbow trout, we strongly suggest that application of these plants is beneficial for immunity and survival rate of fish. So, economical efficacy was promoted.

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