Dietary effect of *Lippia citrodora* essential oil on some hematological, biochemical, growth performance and body composition of *Cyprinus carpio* Linnaeus, 1758

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Abstract

This study was conducted to evaluate the effects of different proportions of lemon bee brush essential oil on growth performance, carcass composition, and hematology and biochemical parameters of common carp (*Cyprinus carpio*). Common carp with an average weight of 8 ± 0.2 g were fed for one month with a diet supplemented with Lemon beebrush essential oil (0.15 and 0.3 ml) and with normal diet as controls. The growth performance, body composition, biochemical and hematological factors were measured on day 30. Results of the present study showed that specimens fed a diet supplemented with Lemon beebrush EO in both dosages noticeably increased final weight ($p<0.05$). Group of fish fed on 0.15 and 0.3 LB in the diet had the lowest level in Hb and hematocrit indices ($P<0.05$). Lipid content was lower in fish fed 0.15 LB supplemented diet and ash was decreased in fish fed both LB supplemented diet.

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Introduction

Aquaculture industry is one of the most important sectors of agriculture in the world. Among all cultivated species, the fresh water fish, common carp, *Cyprinus carpio* has the highest interest as human food in the Far East. Elevating the growth rate and immune response of fish species can significantly affect the benefits of fish culturists. There are many extensive researches on using different synthetic or natural compounds to improve growth and enhance fish health to overcome stressful culture condition (Anderson, 1992). Among different compounds, Medicinal plants have been used in traditional systems to treat many diseases (Bhadauria 2012). Secondary metabolites produced by plants are organic chemicals of high structural diversity, which play different functions including chemotherapeutic, bacteriostatic, bacteriocidal and antimicrobial functions (Purohit & Mathur, 1999). As a result, herbal components received much attention in aquaculture industry during the last decade for different purposes such as growth promotion, immunostimualtion, antiviral, antifungal, antibacterial, aphrodisiac as well as appetite stimulators (Citarasu, 2010; Chakraborty & Hancz, 2011).

Lippia citrodora is a species of flowering plant in the verbena family Verbenaceae, native to western South America and growing in many parts of the middle east and Europe in different countries . Common names include lemon verbena and lemon beebrush. *Aloysia citriodora* extract shows antioxidant properties that could play an important role in modulating GSH-reductase activity in lymphocytes and erythrocytes and protecting plasma from exercise oxidative damage (Carrera-Quintanar, Funes, Viudes, Tur, Micol, Roche & Pons 2010). Based on our best knowledge, there was a few scientific literature on the effects of dietary administration of lemon beebrush in fish species. Recent studies assessing the sedative and anesthetic efficacy of the essential oil of *A. triphylla* in fish showed that it prevents stress induced and increase in cortisol (Parodi, Cunha, Becker, Zeppenfeld, Martins, Koakoski, Barcellos, Heinzmann & Baldisserotto (2014); Zeppenfeld, Toni & Becker 2014). Zeppenfeld Hernández, Santinón, Heinzmann , Da Cunha, Schmidt & Baldisserotto (2015) examined the growth, gut morphology and occurance of endocrine cells in the gastrointestinal tract of silver catfish fed diet supplemented by essential oil of *Aloysa triphyla*.

Oral administration is the only method economically suited to extensive aquaculture and is widely used method, which is more effective than the other methods. It is non stressful and allows mass administration regardless of fish size (Sakai, 1999; Galindo-Villegas & Hosokawa, 2004). Therefore, the present study was performed to determine the effects of dietary lemon beebrush essential oil on some growth and biochemical parameters in common carp.

Materials and Methods

Fish diet preparation

Dried leaves of lemon beebrush were obtained from local shop. An essential oil was prepared according to Zeppenfeld et al (2015) and mixed with commercial diet (Beiza) to achieve 0.15 and 0.3 ml Lemon beebrush (LB) essential oil (EO)/Kg fish. The experiment was performed for 30 days.

Fish

Common carp (N: 180, Weight: 8±0.2g) were purchased from a private farm (Carp Farm, Golestan province, Iran) and were transported to the Gonbad Kavous University, fish health unit. They were distributed in 9 fiberglass tanks (three replicates per group and 20 fish per tank) filled with 100 L water (temperature = 26.5 ºC, dissolved oxygen = 5.2 and PH = 8.01 ppm).

The specimens were fed a commercial diet for 2 weeks to be acclimated to the conditions, and to recover from the stress of transportation.

Data collection and sample analysis

At the end of experiment, the fishes of each aquarium were weighed seprately and average final weight (g/fish) and length (cm) was calculated. After blood sample collection, all fish were scarified and soon the abdominal cavities of three fish from each aquarium were opened to remove viscera which are to be weighed at once.

Chemical analyses of fish

Whole-fish body from each treatment were analyzed according to the standard methods of AOAC (1999) for moisture, crude protein, crude fat and ash.

Growth assessment

Weight , total length, body weight gain (BWI), feed conversion ratio (FCR), specific growth rate (SGR), daily growth rate (DGR) and condition factor (CF) of 30 randomly selected fish in (0.015 & 0.03%) beebrush and control groups were calculated. Final weight and length were measured.

The calculation formula for relative index are listed below:

$$WBI\ (\%) = 100 \times (wf - wi)/wi$$

$$FCR\ (\%) = (\text{dry feed intake (g)}/\text{wet WG (g)}) \times 100$$

$$SGR\ (\%\ day\text{-}1) = (Ln\ wf - Ln\ wi) \times 100/t$$

$$CF\ (\%) = 100 \times [\text{wet weight (g)}/TL3\ (cm)]$$

$$DGR\ (\%) = (wf - wi) \times 100/t$$

wf and wi were final and initial fish weights, respectively; TL was total length and t is the time in days between weighting.

Hematological and serum analysis

The blood was immediately used to determine the number of red blood cells (RBC) and white blood cells (WBC) by means of a haemocytometer slide (Improved Neubauer type) at a magnification of x 400. Haematocrit (Hct) was determined by the microhematocrit method. Haemoglobin (Hb) concentration was conducted by using the cyanohaemoglobin method (Stoskopf 1993). Briefly, 20 µl of

whole blood was mixed with 5 ml of Drabkin's solution (Sigma-Aldrich) in a test tube before allowing to stand for at least 15 min at room temperature. The absorbance (A) was measured at 540 nm. The haemoglobin concentration of the blood sample was calculated from a curve prepared from known standards. Mean corpuscular volume (MCV: Fl), mean corpuscular hemoglobin (MCH: pg) and mean corpuscular hemoglobin concentration (MCHC: %) were measured using the standard routine techniques. Plasma cortisol levels were determined by radioimmunoassay (Grutter & Pankhurst 2000) in samples shipped frozen to the laboratory, using the Diaplas company kit. Plasma levels of glucose were measured using standard spectrophotometric assays (using Parsazmoon kit) and Urea (Chaney & Merbach 1962) by Parsazmoon® kit.

Statistical Analyses

Mean value and standard error (mean ± SE) for each parameter of all treatments was calculated. The results were subjected to a one way analysis of variance. Data was analyzed using SPSS program, Version 16. Differences between means were compared using Bonferoni test at $P < 0.05$ level.

Results

Growth parameters

The Results of growth parameters are shown in table 2. Weight and CF increased in fish fed with both of Lemon beebrush dosage in comparison with control group ($P<0.05$).

Table1 Composition of commercial diet for common carp (5-20 g)

Nutrients	value	Nutrients	value
TVN mg/100 g	<50	Moisture %	<12
Lisin%	1.6-1.8	Crude protein%	32-36
Methionin%	0.42-0.48	Crude lipid%	7-11
Treonin%	1.05-1.25	Ash%	9-13
Phosphorus %	1-1.4	Crude fibre%	2.5-5.5
Gross energy Kcal/Kg	3600-3800		

TVN: Total Volatile Nitrogen

Table 2 Growth performances of fish 30 days after feeding dietary lemon beebrush (LB)

Treatment	BWI (%)	DGR (%)	SGR(%day)	CF (g cm-3)	FCR	Weight(g)	Length(cm)
LB(0.15ml)	3.26±0.95^a	3.26±0.99^a	0.32±0.09^a	116.77±2/00^a	10.52±2.7^a	10.29±0.21^a	8.81±0.04^a
LB (0.3ml)	2.12±0.75^a	2.16±0.75^a	0.2±0.07^a	115.81±3.77^a	9.78±8.9^a	10.45±0.15^a	9.04±0.24^a
Control (0ml)	4.17±1.6^a	3.43±0.81^a	0.4±0.11^a	103.98±4.24^b	8.55±2.6^a	8.88±0.51^b	8.53±0.18^a

Values are mean ± S.E. Values within the same column, not sharing common superscript letters are significantly different ($P< 0.05$, $n=60$).

Hematological property

Hematological parameters are shown in table 2. It shows that Hb and HCT decreased in fish fed with LB in both dosage in comparison with control on 30th days of experiment (P<0.05). No significant difference was observed in RBC, WBC, MCV, MCH and MCHC level between trial groups (P>0.05).

Table 3 Hematological parameters of fish fed diets containing LB after 30 days.

	RBC (10^6mm3)	WBC (10^3mm^3)	HB (g.dl)	HCT (%)	MCV (µm3)	MCH(pg)	MCHC
LB (0.15ml)	0.32 ± 0.06[a]	1.53 ± 0.11[a]	15.63 ± 1.70[a]	8.37 ± 0.48[b]	50.97 ± 5.23[a]	913.95 ± 184.11a	205.99±31.87[a]
LB (0.3ml)	0.28 ± 0.6[a]	1.38 ± .0.13[a]	6.02 ± 1.12[b]	11.82 ± 0.65[b]	71.77 ± 8.40[a]	351 ± 70.85a	146.43±7.32[a]
Control (0ml)	0.41 ± 0.07[a]	1.88 ± 0.29[a]	20.19 ± 3.14[a]	15.37 ± 0.59[a]	67.88 ± 12.38[a]	482.94 ± 55.49a	145±15.08[a]

Values are mean ± S.E. Values within the same column, not sharing common superscript letters are significantly different (P< 0.05, n=60).

Blood biochemical parameters

The changes in Glucose, cortisol and Urea of plasma are shown in table 3. Dietary Lemon beebrush led to a negligible effect on the serum Urea in fish fed with 0.15 LB (p<0.05). Glucose and cortisol level showed no significant change in treatments (P>0.05).

Table 4 Biochemical parameters of fish fed diets containing LB after 30 days.

Treatment	Glu	Urea	Cortisol
LB (0.15ml)	157.67 ± 15.77[a]	15.33 ± 2.84[a]	24.26 ± 0.93[a]
LB (0.3ml)	223.60 ± 23.98[a]	10.20 ± .0.96[b]	25.50 ± 1.23[a]
Control (0ml)	189.80 ± 22.02[a]	8 ± 1.14[b]	21.36 ± 2.41[a]

Values are mean ± S.E. Values within the same column, not sharing common superscript letters are significantly different (P< 0.05, n=60)

Fish body composition

Results of proximate analyses of fish fed the Lemon beebrush and control diets are illustrated in Table 4. DM content in different fish groups ranged between 22.11% (LB 0.3) and 24.27% (LB 0.15) which showed no significant difference in comparison with control group (23.49%). Fish fed LB (0.15) has significantly higher Lipid content (22.06%) when compared with fish fed either LB(0.3) or control diets (19.81 and 20.44%, respectively). Ash analyses indicate a significant decrease in its value in fish fed LB (0.15 & 0.3) diets when compared with control diets. Protein content showed no significant differences between groups.

Table 5 Body compositions of fish fed diets containing 0.15 and 0.3 LB and control after 30 days.

Treatment	DM	Pr	Lipid	Ash
LB (0.15ml)	24.27 ± 0.26^{a}	65.05 ± 0.41^{a}	22.06 ± 0.28^{a}	12.24 ± 0.21^{b}
LB (0.3ml)	22.11 ± 0.46^{a}	$66.93 \pm .0.22^{a}$	19.81 ± 0.28^{b}	12.46 ± 0.08^{b}
Control (0ml)	23.49 ± 0.74^{a}	65.29 ± 0.29^{a}	20.44 ± 0.47^{b}	13.60 ± 0.14^{a}

Values are mean ± S.E. Values within the same column, not sharing common superscript letters are significantly different ($P< 0.05$, n=10).

Discussion

Accumulative effects of chemical additives and its possible threats for human health has made obligatory limits for using these components, as a result, in recent years researchers have tried to introduce natural feed additive such as aromatic and medicinal plants. However, despite of positive achievements of applying herbal medicine in fish diet on their weight gain, physiological changes due to medical herbs use, should not remain hidden. Natural plant products have been reported to promote various activities like anti stress, growth promotion, appetite stimulation, tonic and immunostimulation, and to have aphrodisiac and antimicrobial properties in finfish due to the active principles such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils (Citarasu, Sekar, Babu & Marian (2002); Sivaram, Babu, Citarasu, Immanuel, Murugadass & Marian (2004)). The herbal extracts, *Astragalus membranaceus*, *Portulaca oleracea*, *Flavescent sophora* and *A. paniculata*, act as an anti-stress and induce the immunological parameters such as serum lysozyme activity, SOD, NOS and levels of total serum protein, globulin, and albumin in *Cyprinus carpio* (Wu, Yuan, Shen, Tang, Gong, Li, Sun, Huang & Han 2007).

According to our knowledge, there are a few studies of *Lippia citrodora* essential oil on fish species. Anesthetic activity of the essential oil of *Aloysia triphylla* and effectiveness in reducing stress during transport of albino and gray strains of silver catfish, have been

determined by Thylise, Parodi, Cunha, Alexssandro, Becker, Carla, Zeppenfeld , Dirlaine , Martins , Gessi Koakoski , Leonardo Gil Barcellos , Heinzmann & Baldisserotto (2014). Their research indicated that the EO of *A. triphylla* is an effective anesthetic for albino and gray silver catfish.

The positive effects of different plant components as a growth promoter in aquaculture has been reported previously, e.g. in common carp by using Rheum officinale extract (Xie, Liu, Zhou, Su, He, Pan, Ge, Xu, 2008). Livol (IHF-1000) is an herbal growth promoter containing different plant ingredients such as *Bohaevia diffusa*, *Solanum nigrum*, *Terminaelia arjuna*, Colosynth and black salt has been found to significantly improve digestion, thereby leading to better growth, production and health in cultivable fishes (De Baulney, Quentel, Fournier, Lamour, & Le Gouvello (1996); Shadakshari (1993); Unnikrishnan (1995)). Maheshappa (1993) studied the effect of Livol (IHF-1000) on the rohu, *L. rohita*, the Livol incorporated diet stimulated digestive enzyme activity and led to increased consumption.

In the present study, an improvement in common carp final weight and CF indices was recorded in fish fed diet containing lemon beebrush diet after 30 days but the other measured indices were insignificantly affected among groups. Dietary effect of *Aloysia triphylla* essential oil on fish species has only been determined by Zeppenfeld et al (2015). According to above authors, there was no significant difference for the growth parameters evaluated at 30 days of experiment in silver catfish, which was in contrast with our findings. The highest final weight, RW and SGR were attained after 60 days in fish fed 2.0 mL EO of *A. triphylla* per kg of diet (Zeppenfeld et al, 2015). Most of growth performance in carp species was in high dosage plant supplemented diet. Replacement of fish meal by more than 25% of *spirulina platensis* could significantly increase rohu growth performance (Nandeesha Gangadhar, Varghese & Keshavanath 1998). However, the final weight gain, specific growth rate, food conversion ratio and protein efficiency ratio of common carp were not affected by 25-100% of *Spirulina* supplementation (Nandeesha et al, 1998). Other plant additives, as garlic and ground dried ginger, needed a higher dietary supplementation (10 – 20 g kg^{-1} feed).

Assessment of blood parameters is one of key indices, indicating the physiological statues of fish. Applying herbal supplements has main effect on fish hematology. In present study, Hb and HCT showed considerable decrease in treatments with supplemented diet. The haemoglobin concentration got decreased with increase in concentration of the plant extracts of Mucuna pruriens in lobea rohita (Ojha, Chadha, Sain ,Damroy , Chandraprakash & Sawant 2014) similar to those reported in *C. gariepinus* to cassava effluents and tobacco (*Nicotina tobaccum*) leaf extracts (Adeyemo (2005); Omoniyi, Agbon & Sodunke (2002)) and aqueous leaf extracts of *Lpidagnathis alopecuroides* (Gabriel, Obomanu & Edori 2009). On contrary, a significant increase was found in hemoglobin and hematocrit level in labeo rohita fed diets enriched with mango

kernel powder and garlic (Sahu, Das, Mishra, Pradhan & Sarangi 2007) , in flanders fed with a mixture of herbs including Medicata fermentata, Crataegi fructus, *Artemisia capillaries* and *Cnidium officinale* increased hemoglobin and hematocrit (Ji, Jronh, Gwang-Soon, Lee, Yoo & Takii 2007). Moreover, Dietary effect of *Silybummarianum* extract increased HCT level in common carp (Alishahi, Soltani & Mesbah 2011) and *Zataria multiflora* and *Eucalyptus globolus* essential oil had little effect of hematocrit of common carp (sheikhzadeh, Soltani, Ebrahimzadeh-Mousavi, Shahbazian & Norouzi 2011). The decrease that occurred in the current hematological parameters could be attributed to the fact that the active ingredients in *Aloysia triphylla* play inhibitory roles on the level of these blood indices body.

In aquaculture industry, especially intensive culture, various stressors such as ambient temperature, high stocking density, physicochemical parameters, transport, and confinement induce stress on fish (Chen, Sun, Tsai, Song & Chang 2002). Some Nutritional supplements can alter the levels of cortisol and glucose in the serum of fish which are two key indicators associated to stress response (Li , Burr, Goff, Whiteman, Davise, Vega, Neill & Gatlin (2005) ; Nobahar, Gholipour-Kanani, Kakoolaki & Jafarian 2014). The level of blood glucose abruptly rose in a short time in order to provide the sufficient amount of energy for fish under stress conditions, (Rotllant, Tort, Montero, Pavlidis, Martinez, Wendelaar Bonga & Balm 2003). According to our results, glucose and cortisol have not changed in fish treated with LB essensial oil. Xie et al (2008) showed serum cortisol reduction in stressed common carp treated with anthraquinone extract as an immunostimulant. Moreover, Wendelaar Bonga (1997) reported decrease in glucose and cortisol content in *Megalobrama amblycephala* after temperature stress meanwhile the diet was supplemented with 0.1% Anthraquinone extract or 60 ppm Emodin for 8 weeks . Addition of the 40 and 50 uL L^{-1} EO of *A. triphylla* to the water of transport increased whole-body cortisol levels in silver catfish. On the contrary, the glucose levels were significantly lower in fish transported with EO of *A. triphylla*. Urea level showed increase in 0.15LB treated fish. This raise may be due to kidney dysfunction (Abdelvahab and EL Bahr 2012).

Limited scientific research has been carried out to evaluate the effects of medicinal plant on carcass quality in aquatic animal. Body composition in common carp fed by pellet diet supplemented with LB 0.15 showed significant increase in lipid content. Moreover, in both of LB supplemented diet fish caracass had lower ash content. Common carp body composition in fish fed marshmallow (*Althaea officinalis L.*) extract supplemented diet showed slight changes (Fallahpour, Banaee & Javadzade 2014). Similar results were observed in fish fed with alfa alfa (15 and 20%), soybean meal (30 and 60%) and cottonseed meal (30 and 60%), (Ali, Al-Asgahn, Al-Ogaily & Ali (2003); Toko, Fiogbe & Kestemont (2008)). On contrary, proximate body composition including the levels of moisture, crude protein,

crude lipid and ash as % of wet weight were not affected by inclusion of the plant extract in the diets of Nile tilapia, *Oreochromis niloticus* (Abdel, Mostafa, Ahmad, Mousallamy, Samir 2009), red sea bream (Ji et al., 2007). Probably, these differences are due to environmental factors such as temperature (Cordier, Brichon, Weber & Zwingelstein (2002); Tocher, Fonseca-Madrigal, Dick, Ng , Bell & Campbell (2004)) pH and salinity which influence lipid content in fish (De Torrengo & Brenner 1976). In addition, different climate, age and weight of samples have determining effect. In addition, Plant composition as well as the duration of the experiments can affect the response (Citarasu, 2010).

Zeppenfeld et al (2015) reported no significant differences between cat fish fed *A triphylla* EO with regard to the epithelial area. However, the number of folds was higher in fish fed a diet supplemented with 2.0 mL *A. triphylla* EO per kg (Zeppenfeld et al 2015). According to above researchers, the presence of more folds is an indication of enhanced nutrients and electrolytes absorption capacity (Nabuurs (1995); Branco, Soares, Bretas, Cabral, Vieites, Bonaparte & Mota (2010)), this increase may be greater due to higher turnover rate caused by stimuli resulting from the action of active principles of plants and their EOs that promote the rapid growth of villous (Branco et al.2010). Various plant extracts from herbs and spices are reported to improve animal performance by stimulating action on gut secretions or by having a direct bactericidal effect on gut. It seems that, an improvement in the physiological or metabolic status is more responsible for the higher final weight in fish fed 0.15 and 0.3 ml LB/ kg of diet than the diet itself (Escaffre, Kaushik & Mambrini 2007).

In conclusion, the addition of 0.3 mL LB per kg to diet can improve growth performance of common carp. However, its dietary effect on some hematological factors was not encouraging.

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اثر رژیم غذایی اسانس *Lippia citrodora* بر برخی از عملکرد هماتولوژیک، بیوشیمیایی، عملکرد رشد و ترکیب بدن *Cyprinus carpio*

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چکیده

این تحقیق به منظور بررسی تاثیر نسبت های مختلف روغن اسانس بره بر روی عملکرد رشد، ترکیب لاشه و هماتولوژی و پارامترهای بیوشیمیایی کپور معمولی (*Cyprinus carpio*) انجام شد. کپور معمولی با وزن متوسط ۲ ± ۸ گرم برای یک ماه با رژیم غذایی حاوی اسانس مارچوبه (۰,۱۵ و ۰,۳ میلی لیتر) و رژیم غذایی معمول به عنوان شاهد تغذیه شد. عملکرد روزانه، ترکیب بدن، عوامل بیوشیمیایی و هماتولوژیک در روز ۳۰ اندازه گیری شد. نتایج مطالعه حاضر نشان داد که نمونه های تغذیه شده با رژیم غذایی حاوی Beebrush EO در هر دو دوز قابل توجه افزایش وزن نهایی (۰/۰۵ p >). گروهی از ماهی های خوراکی با میزان ۰,۱۵ و ۰,۳ LB در رژیم غذایی پایین ترین سطح در شاخص های Hb و هماتوکریت داشتند ($P <0.05$). محتوای لیپید در ماهی خوراکی ۰,۱۵ لیتر خوراکی و رژیم غذایی افزوده شده ماهیان خاویاری کاهش یافت.

کلمات کلیدی: مارچوبه، هماتولوژی، رشد، کپور

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