Effect of dietary wood betony, *Stachys lavandulifolia* extract on growth performance, haematological and biochemical parameters of common carp, *Cyprinus carpio*

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Abstract

A 6 week study was conducted to assess the effects of wood betony (WB), *Stachys lavandulifolia* extract on growth performance, hematological and biochemical parameters of common carp, *Cyprinus carpio*. Different levels of the WB extract (0, 2, 4 and 8 % weight per weight, W/W, 0WB, 2WB, 4WB and 8WB) in the diet were used. The results showed that final weight and weight gain were significantly improved by WB (p<0.05), while other growth parameters such as food conversion efficiency and condition factor did not change (p>0.05). There were no significant differences in hemoglobin, hematocrit, mean erythrocytes of hemoglobin, mean erythrocyte volume, mean hemoglobin erythrocyte concentration and white blood cell (WBC) counts (p>0.05), while, red blood cells (RBC) counts showed significant declining trend by increasing the level of the plant extract from control to 8WB (p<0.05). Significant elevation in the levels of total protein, albumin and globulin and albumin/globulin ratio by increasing WB concentration in the diet were observed (p<0.05). Diet enriched by WB could decrease serum level of triglycerides and cholesterol in comparison with the control (p<0.05). Based on the results of this study, it could be concluded that feeding common carp with WB can improve growth and some immunity characteristics as well as lipid metabolism.

Keywords: Medicinal herb, Immunity, Lipid metabolism, Common carp, *Cyprinus carpio.*

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Introduction

Fish cultivation under intensive conditions has been increased over the past decades all around the world. Intensive aquaculture practices may have significant adverse effects on fish welfare because of different kind of culture conditions and husbandry activities such as grading, handling, transportation, as well as poor culture conditions which increase the risk of fish disease. Different practices such as improving culture condition, using optimal fish density, enhancing fish immunity by natural and or synthetic immune-stimulants could be done to reduce the risk. Food additive is now in the increase mostly because of the adverse effects of synthetic components such as bioaccumulation and environmental pollution, generating resistant pathogens as well as their high cost imposed to aquaculture practitioners (Chakraborty and Hancz, 2011). Several herbal components such as flowers, leaves, seeds and roots from different plant species have been shown to enhance growth, non-specific immune system, stress response as well as survival rates of cultivated species like African catfish, *Clarias gariepinus* (Dada and Ikuerowo, 2009; Soosean *et al.,* 2010), Tilapia *Orechromis mossambicus* (Immanuel *et al.,* 2009), Common Carp, *C. carpio* (Alishahi *et al.,* 2010; Pakravan *et al.,* 2012), and Olive flounder, *Paralichthys olivaceus* (Cho and Lee, 2012). WB, *Stachys lavandifulia* Vahl belongs to family Lamiaceae, is grown in many parts of Iran, Iraq, Turkey, Syria, Armenia as well as Georgia (Javidnia *et al.,* 2004). Fresh and dried aerial parts such as leaves and flowers, as well as roots have been used as traditional drugs for treatment of wounds and bruises, mouth ulcers, gum inflammations (Ody, 1997) and treating arthritis and respiratory inflammatory disorders (Rezazadeh *et al.,* 2009). Alkanoids (including stachydrine and trigonelline), tannins, saponines, nicotinic acid and steroids are the main components of WB (Vundac *et al.,* 2007; Soleimani, 2011; Bahrami Babaheydari *et al.,* 2013) and some of them have shown a variety of biological effects on *Vibrio* sp. (Ghasemi Pirbalouti *et al.,* 2011). The biological activity of WB has not yet been studied in fish. Hence the present study was aimed to evaluate the effects of dietary inclusion of WB extract on growth performance, haematological and biochemical characteristics of common carp juveniles as one of the most important cultivated fish species in the world.

Materials and methods

Fish

The juvenile common carp, *C.carpio* (44± 0.62) were obtained from a fish propagation and breeding center in Isfahan, Iran. Fish were kept under the same environmental conditions, placed in 10 m^3 rectangular concrete tank for 2 weeks for acclimatisation. They were fed a commercial carp food (Isfahan Mokkamel, Iran). The proximate composition of the commercial diet (wet basis %) were 9.2% humidity, 32% protein, 10.2% lipid and 11.1%

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ash (based on our analysis, data not shown).

Plant extract

In mid-spring, 2012, the WB aerial parts including flowers and leaves were collected from natural habitat in Isfahan Province, Iran. Hydro-alcoholic plant extraction was done based on Ghasemi Pirbalouti *et al.* (2010) with some modification. Briefly different aerial parts of the plants were washed thoroughly with distilled water and dried in room temperature under shading; and finally the plants were ground into powder. One hundred g of powdered plant material was soaked in 500 mL of ethanol (75%) for 48 hrs, shacked vigorously to allow for proper extraction. After filtering of the extract through Whatman No. 1 paper, the filtrate was concentrated using a rotary evaporator at around 50˚C. Finally 20 mL of concentrated extract was obtained from l00 g of the plant powder (each ml ~ 5 g). The chemical composition of the plant extract was determined using Agilent Technologies 7890A (USA) with HB5 at Central laboratory of Isfahan University of Technology (Bahrami Babaheydari *et al*., 2013).

Feed preparation and feeding trail

A basic commercial carp diet was purchased from Isfahan Mokkamel, Isfahan, Iran. The food was ground into powder. One hundred mL of distilled water were added to the basic food powder and were made into noodles, using noodle-making machine (1 mm diameter), to make a control diet (0WB). For plant extracted added diet, 4, 8 and 16mL of the concentrated plant extract were added to water, the final volume were adjusted to 100 mL and used for making diet containing 2, 4 and 8% WB extract (defined as 2WB, 4WB and 8WB), respectively. The noodles were dried at room temperature till the moisture content was reduced below 10%, were broken to very small pieces, packed in airtight plastic containers and kept at 4˚C during the study.

After 2 weeks of acclimatization, the fish were randomly divided into four experimental groups, 0WB, 2WB, 4WB and 8WB as 0, 2, 4 and 8% weigh per weight (w/w) of WB extract in the diet respectively in three replicates. Each replicate contained 15 individuals in a fibreglass tank (110L water volume, 50% renewed each day). Water quality was monitored throughout the experimental days at weekly intervals; temperature 25±1°C, pH 7.21±0.5 and dissolved oxygen concentration 7.5±0.06mg/L. Fish were fed at the rate of 2% of their body weight per day in the period of the experiment for 6 weeks. The daily ration was divided into three meals at 09:00, 14:00 and 19:00 hours. The amount of given feed were readjusted every two weeks based on the fish weight. At the end of the experiment, fish final weight, mean weight gain (MWG), specific growth rate (SGR), average daily growth rate (ADG) and feed conversion ratio (FCR) were estimated for both control and experimental groups. The following

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formula were used to calculate the growth parameters (Soosean *et al.*, 2010).

MWG=(Mean final weight – Mean initial weight);

SGR (%/day)= 100 × [(lnW1 – ln W0) / t], where W0 and W1 are average initial and final body weights, respectively, and t is time (days);

ADG (g/day) = Growth / Experimental duration;

FCR = Food consumed (g) / Weight gain (g);

CF (g/cm^3) = Weight (g) / [Length (cm)]^3.

Blood sampling

At least 9 individuals, 3 fish from each replicate, from each treatment were anaesthetized with clove powder (100 ppm) at the end of the experiment, day 42. Blood sampling (1.5-2 mL) was performed individually from caudal vein (G.18 needle). Half of each sample was placed in heparinised 1.5 vials for haematological analysis and the rest were used for biochemical tests.

Haematology

RBC and WBC were counted manually, using Neubaur haemocytometer (Paul Marienfeld Gmbh, Lauda, Koenigshofen, Germany) after the blood was diluted (200 times for RBC and 50 times for WBC) with Diace solution containing brilliant cresyl blue 0.1 g, sodium citrate 3.8 g, and formaldehyde 37% 0.2mL in 100mL distilled water (Dorafshan *et al.*, 2008). Haematocrit (Hct%) was measured using heparinised microhaematocrit capillary tubes after centrifugation (2500 rpm for 5 min). Haemoglobin concentration (Hb: g/dL) was measured using cyanomethemoglobin spectrophotometrically. Differential WBC counts (lymphocyte, monocyte and neutrophil portions as WBC%) were determined using blood smears under a light microscope according to Houston (1990). Mean erythrocytic haemoglobin (MCH), volume (MCV), and haemoglobin concentration (MCHC) were calculated using below equations (Dorafshan *et al.*, 2008).

MCHC (g/dL) = Hb (g/dL)/Hct ×100

MCH (g/dL) = Hb (g/dL)/RBC (10^6 mm^3)

MCV (nm^3) = Hct (%) ×10/RBC (10^6 mm^3)

Blood serum biochemistry analysis

The blood samples were kept at 4°C for about 4 hours to clot. The tubes were then centrifuged at 3000 rpm for 10 min and the supernatant serum was collected. The serum was kept frozen at -20°C until analysis for total protein (Tietz, 1986), albumin (Doumas *et al.*, 1977), globulin (total protein–albumin; g/dl) and albumin: globulin (A:G) ratio (Kumar *et al.*, 2005). Triglycerides and cholesterol analyses were done based on method described by Davidson and Nelson (1977). All these measurements were made in duplicate for verification.

Statistical analysis

Statistical analysis was performed by one way ANOVA at 5% significant level. A multiple comparison test (Duncan multiple range test, DMRT)

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was conducted to compare the significant differences among the groups using SPSS V.19. Values are presented as mean±standard deviation.

Results

Chemical composition of WB extract

Totally, about 39 different components have been detected in the extract. The main chemicals are phenol and its derivates (12.54%), sabinene (7.37), β-pinene (5.14%), myrcene (5.12%), 2-Furancarboxaldehyde (5.11%), 1-Methyl-pyrrolidine-2-carboxylic (5.14), pyran (4.21%) and myristicine (4.12%). Some basophilic chemicals were also observed in the extract such as enoic acid (1.58%), benzoic acid (1.52%) and acetic acid (1.02%).

Growth performance

The growth responses (such as final weight, weight gain and FCR) of juvenile common carp are presented in Table 1. Dietary WB could cause significant changes in the final weight and weight gain, where the highest final weight (69.25±2.31g) and weight gain (25.22±1.01g) of 8WB was significantly (p<0.05) higher than that reported for the fish fed on the control diet, but was not significantly changed in comparison to 2WB and 4WB (p>0.05; Table 1). FCR was in the range 2.31-2.77, showed insignificant improvement by elevating WB doses while SGR and ADG were measured in the range 0.85-0.99 (%/day) and 0.49-0.53 (g/day), respectively and showed insignificant increase from 0WB to 8WB (p>0.05; Table 1). No significant differences was noticed for final condition factors (CF) of juvenile common carp in all the dietary treatments used in the present study (p>0.05; Table 1).

Table 1: Growth performance and feed utilization of juvenile common carp fed with diets contacting various percentages of wood betony extract for 6 weeks.

Variable (units)	0WB	2WB	4WB	8WB
Initial weight (g)	43.88 ± 0.34^{a}	43.42 ± 0.68^{a}	44.53 ± 0.42^{a}	44.02 ± 1.52^{a}
Final weight (g)	57.61 ± 0.62^{a}	63.29 ± 3.31^{ab}	65.73 ± 2.02^{ab}	69.25 ± 2.31^{b}
Weight gain (g)	13.73 ± 0.85^{a}	19.87 ± 2.09^{ab}	21.20 ± 2.43^{ab}	25.22 ± 1.01^{b}
FCR	2.77 ± 0.01^{a}	2.59 ± 0.27^{a}	2.69 ± 0.31^{a}	2.31 ± 0.11^{a}
SGR (%/day)	0.85 ± 0.01^{a}	0.90 ± 0.07^{a}	0.86 ± 0.07^{a}	0.99 ± 0.6^{a}
ADG (g/day)	0.49 ± 0.18^{a}	0.54 ± 0.18^{a}	0.52 ± 0.22^{a}	0.53 ± 0.20^{a}
CF (g/cm^3)	1.55 ± 0.24^{a}	1.57 ± 0.17^{a}	1.52 ± 0.28^{a}	1.58 ± 0.28^{a}

Values are expressed as mean±SD.

Mean with the same letter in the same row is not significantly different (p<0.05).

WB: Wood Betony, FCR: Feed Conversion Ratio , SGR: Specific Growth Rate, ADG: Average Daily Growth Rate, CF: Condition Factors.

Hematology

Table 2 shows the haematological parameters of the fish with the groups fed with the experimental diets. The Hb and Hct were in the range 9.23-11.23 g/dL and 27.14-35.01%, respectively without any significant differences among groups (p>0.05). The highest mean value of WBC (cell/mm^3) was measured at 8WB followed by 2WB, 4WB and 0WB, but no significant difference was observed (p>0.05).

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Lymphocyte, monocyte and neutrophil portions were the most frequent WBC in all tested fish, respectively, Although an increasing trend in Lym% of WBC was observed by elevating the plant extract doses (Table 2), there were no significant differences among groups regarding these parameters (p>0.05). The final values reported for MCH (g/dL), MCV (mm^3) and MCHC (g/dL) as secondary hematological parameters highly varied but no significant differences were observed (Table 2; p>0.05). The 8WB and 4WB groups of fish had the lowest level of RBC, while the highest values were observed in 2WB and then 0WB, It means by increasing dietary hydroalcoholic extract of WB levels to 4 and 8% of the diet, the number of RBC decreased significantly in comparison to 2WB and 0WB (p<0.05; Table 2). The highest WBC/RBC×10^{-2} ratio (0.11±0.006) was measured at 8WB followed by 4WB which was significantly higher than the values in the 0WB and 2WB (p>0.05; Table 2).

Biochemical analysis

Serum total protein, albumin, and globulin contents increased significantly (p<0.05) in fish fed on 4 and 8 % of WB compared to those fed on control (0WB) or 2WB (Table 3). The A:G ratio showed significant increase by elevating the WB percentage in the diet, where the highest ratio (0.65±0.16) was measured in 8WB (Table 3). Serum triglycerides and cholesterol were significantly affected by dietary WB (p<0.05; Table 3). Diets containing WB caused significant decline of triglycerides in comparison to control (p<0.05; Table 3). The lowest cholesterol contents were calculated for 2 and 4WB (p<0.05; Table 3), While there were no significant differences in cholesterol level between the control (0WB) and 8WB groups after 6 weeks of the experiment (p<0.05; Table 3).

Table 2: Hematological parameters of fish fed with diet containing different levels of wood betony extract.

Variable (units)	0WB	2WB	4WB	8WB
Hb (g/dL)	10.23 ± 0.75^a	11.23 ± 0.75^a	9.23 ± 1.70^a	9.83 ± 1.25^a
Hct (%)	33.11 ± 3^a	35.01 ± 2^a	30.66 ± 6.02^a	27.14 ± 1^a
RBC (cell/mm^3 × 10^6)	2.44 ± 0.03^a	2.53 ± 0.01^a	2.16 ± 0.01^b	2.16 ± 0.07^b
WBC (cell/mm^3)	1958 ± 82^a	2131 ± 132^a	2076 ± 258^a	2345 ± 181^a
MCH (g/dL)	41.60 ± 3.05^a	41.64 ± 2.78^a	45.84 ± 8.45	43.74 ± 5.60^a
MCV (nm^3)	134.16 ± 12.19^a	129.74 ± 7.41^a	152.25 ± 29.92^a	120.22 ± 4.45^a
MCHC (g/dL)	31.26 ± 4.68^a	32.12 ± 2.02^a	30.55 ± 6.03^a	36.51 ± 5.45^a
Lym (% of WBC)	75.6 ± 8.5^a	76.6 ± 10.2^a	78.3 ± 3^a	80.6 ± 9.2^a
Mon (% of WBC)	21.3 ± 7^a	20.3 ± 10^a	18 ± 2.6^a	17 ± 8.5^a
Neu (% of WBC)	3.3 ± 1.5^a	3 ± 1^a	3.6 ± 1.5^a	2.6 ± 2^a
WBC/RBC × 10^{-2}	0.08 ± 0.004^a	0.08 ± 0.008^{ab}	0.10 ± 0.009^{bc}	0.11 ± 0.006^c

Values are expressed as mean±SD.
Mean with the same letter in the same row is not significantly different (p<0.05).
WB: Wood Betony, Hb: Haemoglobin Concentration, Hct: Haematocrit, RBC: Red Blood Cells, WBC: white Blood Cells, MCV: Mean Cell Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Cell Hemoglobin Concentration.

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Table 3: Biochemical parameters of fish fed with diet containing different levels of wood betony extract.

Variable (units)	0WB	2WB	4WB	8WB
T.pro (g/dL)	3.10 ± 0.18^{a}	3.24 ± 0.31^{a}	4.05 ± 0.15^{b}	4.45 ± 1.04^{b}
Alb (g/dL)	1.08 ± 0.10^{a}	1.35 ± 0.12^{a}	1.62 ± 0.15^{ab}	2.05 ± 1.07^{b}
Glb (g/dL)	2.01 ± 0.15^{a}	1.88 ± 0.33^{a}	2.43 ± 0.22^{b}	2.40 ± 0.3^{b}
Alb:Glb	0.54 ± 0.07^{a}	0.60 ± 0.16^{ab}	0.61 ± 0.12^{ab}	0.65 ± 0.48^{b}
Tg (mg/dL)	521.66 ± 21^{b}	310.66 ± 102^{a}	281.44 ± 83^{a}	352.22 ± 140^{a}
Chol (mg/dL)	182.77 ± 32^{b}	139.11 ± 25^{a}	139.88 ± 33^{a}	163.12 ± 29^{ab}

Values are expressed as mean±SD.
Mean with the same letter in the same row is not significantly different (p<0.05).
WB: Wood Betony

Discussion

In recent decades, the active area of research on fish culture has been the use of a variety of feed additives for different purposes such as growth promoters and immunostimulat. From these additives, plants and their derivatives have a key role, mainly because of the continued consumer pressure about a ban on synthetic compounds and antibiotics in different countries (Ajiboye *et al.*, 2012). Plant based components are mostly biodegradable, safe for human and environment, biocompatible and usually much cheaper than synthetic compounds (Immanuel *et al.*, 2009). Several herbal compounds were tested in aquaculture activities for different aquatic species and mainly finfish such as carps, cichlids and trout (for review see Chakraborty and Hancz, 2011).

Despite the traditional use of WB as an herbal drug in eastern countries, especially the Middle East, to the best of our knowledge, no study has been conducted on using WB extract as feed additive material in aquaculture yet. So, the present experiment focused on the oral administration of WB extract to common carp, as one of the most important cultivated fish species all around the world.

Growth rates, defined as final weight and weight gain were significantly affected by experimental diets (p<0.05), while there were no significant changes on some other growth performance parameters such as FCR, SGR and ADG (p>0.05). It has been reported that different plant additives can enhance growth rate in different fish species such as African catfish *C.gariepinus* brood stock (Dada and Ikuerowo, 2009) and fingerling (Soosean *et al.*, 2010). Unlike these reports, the dietary inclusion of some plant extracts has had not much impact on growth response as indicated in juvenile pikeperch, *Sander lucioperca* fed on two medicinal herbs *Astragalus radix* and *Lonicera japonica* (Zakęś *et al.*, 2008) and common carp which received willow herb, *Epilobium hirsutum* (Pakravan *et al.*, 2012). These differences could be explained by the different species of plants, the method of administration, extraction, the aquatic species in question and even culture conditions (Alishahi *et al.*, 2010). Another factor which may impact the effectiveness of the herbal adjuvant as a growth promoter is the

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duration which the diet is applied, for example while the immunostimulatory effects of herbal extracts on the diet is apparent after a 2-4 week treatment, the positive impact on growth rate was noted following 8-12 weeks in red sea bream, *Pagrus major* and Japanese flounder, *P. olivaceus*, respectively (Ji *et al.,* 2007a, b cited in Zakęś *et al.,* 2008).

There is no exact explanation for the efficiency of medicinal herbs on growth performance of different fish species. Kim *et al*. (1998) reported that unknown factors in the plants can affect growth rates. It is also possible that presence of some bioflavonoid with estrogenic activity can promote growth rate (Kocour *et al.,* 2005; Dada, 2012). It is also well documented that different antibiotics can be used as growth promoter in different animals such as cows, broilers and fish maybe due to alternation of the normal intestinal microbes resulting in more efficient digestion, pathogen and disease suppression and immune system release (Phillips *et al.,* 2004). Based on the WB extract analysis, phenol and their derivates, β-pinene, saponin, sabinene and myrcene are the major components of WB extract. Several studies have been showed that these chemicals can inhibit growth of potential infections from different pathogens including bacteria, fungus and even viruses (Francis *et al.,* 2002; Leite *et al.,* 2007; El-Serafy *et al.,* 2009). On the other hand, the antimicrobial activities of different plants belonging to *Stachys* genus including *S.lavandulifolia* have been reported before (Asadi *et al.,* 2010; Ghasemi Pirbalouti *et al.,* 2012; Benmebarek *et al.,* 2013). So, the positive effect of WB extract diets on growth performance of common carp can be related to its antimicrobial activity. However, further studies are required to find out the exact mechanisms.

The haematological indices are a useful index, reflecting culture conditions such as the effects of dietary treatments on fish welfare, stress responses or as a diagnostic characteristic for some infectious diseases (Houston, 1990). The erythrocyte count was lower in 4WB and 8WB groups when compared to control (0WB) and 2WB, which might indicate the adverse effects of high dose of WB on haematopoietic tissue of common carp. There have been some conflicting results about RBC changes after phytochemical use in the fish diets. While a significant increase has been reported in RBC counts in the channel catfish, *Ichthalurus punctatus* (Duncan and Klesius, 1996), Rohu carp, an Indian major carp, *Labeo rohita* juvenile (Sahu *et al.,* 2007) and African catfish fingerling (Soosean *et al.,* 2010), there were no significant changes in RBC (Dada and Ikuerowo, 2009; Alishahi *et al.,* 2010) or even RBC reduction (Pakravan *et al.,* 2012) after dietary herbal administration were observed. In the present study the haemoglobin concentration was not significantly different from control group which may indicate feeding fish with diet containing different levels of

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WB as a feed additive, did not impose any kind of stress. It is scientifically acceptable that under stressful condition, there will be an increase in releasing of immature RBC_s from head kidney as a haematopoietic tissue, which can cause elevation in haemoglobin concentration in blood (Misra *et al.,* 2006).

Although, there were no significant changes in WBC counts and kinds (as defined by Lym, Mon. and Nut.) between different experimental groups (p>0.05), a very clear increase in WBC in WBC/RBC ratio was observed, coincided with elevating WB extract dose in the diet of common carp which can be considered as an improvement of non-specific immunity in the fish. Too many studies has reported the positive effect of herbal extracts on non-specific immunity of cultivated aquatic species as defined by enhancing in survival after challenge test (Immanuel *et al.,* 2009), Lysozyme activity (Alishahi *et al.,* 2010) and WBC counts (Pakravan *et al.,* 2012). To the best of our knowledge, a few studies have reported the effects of oral administration of herbal extract on serum biochemical characteristics of aquatics. Total protein as well as their major components, albumin and globulin have a key role in immune system activity in different species including fish (Siwicki *et al.,* 1994; Kumar *et al.,* 2005). Our results confirmed positive significant effects of WB extract diet on elevating total protein and their component, albumin, globulin as well as A:G ratio in carp. Although certain herbal extracts have shown positive effects on increasing total protein as well as their component as reported for rohu, *L. rohita* (Vasudeva *et al.,* 2004), tilapia *Oreochromis mossambicus* (Immanuel *et al.,* 2009) and common carp (Alishahi *et al.,* 2010), other spices have not done so in rainbow trout *Oncorhynchus mykiss* (Ispir and Mustafa, 2005). The increase in total protein content is usually supported by elevating in WBC counts as a major source of serum protein (Misra *et al.,* 2006). However, because the administration of WB resulted in an insignificant increase in WBC counts, the mechanism for such an effect has not been clearly described and further studies are required.

In this study, triglycerides and cholesterol were reduced by WB administration, while similar results were observed by using four medicinal plants Bermuda grass, *Cynodon dactylon*, deal, *Aegle marmelos*, winter cherry, *Withania somnifera* and ginger, *Zingiber officinale* (1% w/w) on Mozambican tilapia (Immanuel *et al.,* 2009). Feeding aquatics with diets containing phytochemicals can affect fat metabolism (Ji *et al.,* 2007a,b). Some such mechanism maybe active in the common carp feeding on WB diets which helped the fish to utilize lipid effectively as a source of energy, which means that other sources of energy like protein can be used more effectively for somatic growth (Zakęś *et al.,* 2008).

It may be concluded that WB *S. lavandulifolia* can act as a growth promoter and an immunostimulant

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agent (based on serum protein content) in common carp in order to improve aquaculture production. We recommend to evaluate the effects of this extract in more detail emphasizing on hematological changes and the difference between major fish species.

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