Dietary effect of Lemon Verbena (*Aloysia triphylla*) extract on growth performance, some haematological, biochemical, and non-specific immunity and stocking density challenge of rainbow trout juveniles (*Oncorhynchus mykiss*)

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**ABSTRACT**

An eight-week feeding trial was conducted to evaluate the effect of lemon verbena (*Aloysia triphylla*) extract on growth performance, some haematological, biochemical, and non-specific immunity parameters as well as stocking density challenge of rainbow trout (*Oncorhynchus mykiss*). After adaptation, the fish, with an average weight of 25.52 ± 1.31 g, were fed four different dietary lemon verbena inclusion levels (0, 2.5, 5, and 7 g/kg) for six weeks. In the next step, the number of fish in each tank tripled to expose the fish to density challenges for two weeks. There were differences in the final weight, (SGR), (FCR), (PER), and feed intake between the experimental and control groups (*p* < 0.05). The results showed that the cortisol content decreased (*p* < 0.05). The highest levels of FCR, feed intake, plasma triglyceride, cholesterol, (LDL), and (HDL) were observed in the control group. Furthermore, under high stocking density, treatments showed a significant decrease in glucose and cortisol (*p* < 0.05). A significant increase was also shown in (IgM), (RBA), and (SOD) in all treatments. Finally, using lemon verbena extract, up to 7 g/kg of food can be recommended for rainbow trout diets because it can improve growth performance and non-specific immunity parameters.

**Introduction**

Due to the ever-growing human population in the world, the demand for high-quality protein has increased and it seems necessary to identify products that potentially promote the efficiency of fish production (Aanyu 2016). The aquaculture industry has shown interest in using intensive and super-intensive culture systems to produce aquaculture productions, resulting in a widespread of pathogens and infectious diseases, which lead to significant economic losses. Since diseases are among the crucial factors limiting the expansion of aquaculture (Direkbusarakom 2004), various chemical compositions have been employed for the treatment or prevention of these diseases. However, excessive use of antibiotics can create resistant strains of bacteria against antibiotics. As a result, it may negatively affect the treatment of fish, causing zoonotic diseases shared between humans and fish, and damage the environment and fish farms (Cristea et al. 2012).

Improved growth rate and enhanced immune response of different fish species can remarkably in the amount of production in fish farms. A large body of studies has been conducted on the application of different synthetic or natural compounds to improve fish growth and health and overcome stressful culture conditions (Bohlouli et al. 2016). Medicinal plants are among the bioactive compound sources, traditionally used for treating many diseases (Bhadauria 2012). During the last years, herbal derivatives have received much attention in the aquaculture industry, due to their uses for different purposes: growth promotion, immunostimulant (Zargar 2019), antiviral, antifungal and antibacterial activities, appetite stimulator (Citarasu 2010), as well as the diagnosis and control of fish and shellfish diseases (Reverter et al. 2017). *Aloysia triphylla* belongs to the Verbenaceae family and is commonly used as a palliative and digestive aid and treatment for rheum (Zeppenfeld et al. 2016, 2017). It also has anaesthetic and sedative effects on fish (Zeppenfeld et al. 2014). In fact, Phenolic and Terpenoid compositions of *A. triphylla* extract promote growth and immunity in Silver Catfish (Zeppenfeld et al. 2014) and Common Carp (Gholipour-Khani et al. 2017). Rainbow trout (*Oncorhynchus mykiss* (*O. mykiss*)) due to its size and availability, may act as an appropriate research model for alternative and advanced techniques in studies on fish nutrition. Some research has focused on using lemon verbena extract in fish (Zeppenfeld et al. 2014; 2017). Lots of research has represented the effect of many herbal extracts and essential oils on growth performance, immune response, and disease resistance of rainbow trout (Acar et al. 2018; Taheri Mirghaed et al. 2018; Parrino et al. 2019; Zargar 2019; Hassanalizadeh Chari et al. 2020), while a few studies were conducted on the effects of lemon verbena in aquaculture. Haematological and biochemical parameters can help biologists to understand fish homeostasis. As powerful diagnostic tools, they can be used to monitor the health status of fish in response to changes related to nutrition, water quality, and disease in response to therapy (Fazio et al. 2018; Fazio 2019; Ahmed 2020; Bababalaian et al. 2020). In the present study, the effects of oral lemon verbena extract were investigated on haematological, biochemical, and non-specific...
immunity parameters of rainbow trout during crowding stress. To do so, haematological indices, biochemical parameters (cortisol, triglyceride, glucose, cholesterol, LDL, HDL, albumin, Total Protein, AST, ALP, and ALT), and immunological indices (Lysozyme, SOD, IgM, RBA, and alternative complement (ACH50)) were measured before and after crowding stress.

**Material and methods**

**Fish**

The rainbow trout fish were obtained from the local fish culture centre (Gorgan, Iran) and acclimated for two weeks. All fish were clinically healthy and there were no lesions on their body. The fish with the initial average weight of 25.52 ± 1.31 (g) and the average length of 11.64 ± 1.03 (cm) (mean ± SD) were randomly distributed into 300-liter fiberglass tanks (20 fish per tank).

**Fish rearing**

After two weeks of adaptation, the fish were divided into four groups (control and experimental groups named E1-E3), each group was triplicated and 20 fish were assigned to each tank. The fish were fed twice a day, based on 2.5% of their body weight for eight weeks (Aanyu 2016) and they were exposed to 12-hour light during a day. Fish biometry was performed every two weeks and the percentage of the daily meals was calculated based on the biometry. Water quality parameters were monitored during the experimental period. Temperature, dissolved oxygen, and pH were measured by a water checker (Horiba u 10, Japan), averaging 15 ± 1.2°C, 7.7 ± 0.3 mg L⁻¹, and 7.35 ± 0.1 in all treatments, respectively.

**Herbal extract preparation**

Lemon verbena (A. triphylla) hydroalcoholic extract was purchased from Giah Essence Company, Gorgan, Golestan province, Iran. The extract was dried for 72 h (−50°C) by a freeze-drier (Beta LD pluse, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The dried product was pulverized again and kept at 4°C until use (Bohlouli et al. 2016).

**Feeding**

Fish were fed with the control diet for two weeks: a commercial diet (Faradaneh, Iran) with a proximate composition of (dry basis %) contained moisture (10.2%), protein (41%), lipid (14.1%), and ash (7.2%). To make the diets for the three experimental groups (E1, E2, and E3), the commercial diet was moisturized with 400 ml/kg water. Different levels of plant extract (2.5, 5, and 7 g/kg of diet, respectively) were added to the resultant dough, which passed through a mesh (3 mm, meat grinder) and sticks were dried against a fan blow. The sticks were crushed in an appropriate size and kept at 4°C until use (Bohlouli et al. 2016).

**Growth performance**

In order to analyze the growth indices of the juveniles, some biometric parameters of all fish in each tank were measured during the experiment, once every 15 days at least 12 h after the last feeding. At the end of the feeding trial, the fish feed intake (FI), weight gain (WG%), specific growth rate (SGR; % day⁻¹), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate were calculated according to the following formulas (Akrami et al. 2015b):

\[
WG = 100 \times \frac{(W_f-W_i)}{W_i}
\]

\[
FCR = \frac{\text{dry feed intake (g/wet WG(g))}}{\text{protein intake (g)}}
\]

\[
SGR (\%\text{day}^{-1}) = \left(\frac{W_f - W_i}{W_i \times t}\right) \times 100
\]

\[
PER = \frac{WG}{\text{Protein intake (g)}}
\]

\[
\text{Condition Factor (CF)} = \frac{100 \times (\text{final fish weight (g)/final fish length (cm)})^3}{B_k 
\]

where \(W_i\) and \(W_f\) are final and initial body weights respectively and \(t\) is time in days.

**Blood sampling**

At the end of the trial period, three fish were sampled from each replicate and then, anaesthesized using clove solution (300 mg/l). In the next step, the fish blood was taken by a syringe from the caudal vein and the blood samples from each replicate were added to both heparinized and non-heparinized microtubes in order to perform both haematological and immunological studies, respectively.

Blood sera were obtained by centrifuging blood samples at 3000 (rpm) (15,609 g) for 10 min using a Heraeus Labofuge 400, and the sera were removed with a disposable transfer pipette (Shahsavani et al. 2010) and stored at −20°C until analysis for biochemical and immunological studies.

**Haematology**

After diluting blood samples by adding Daice solution, which was made based on laboratory methods of fish pathology (Roberts 1989), the numbers of red blood cells (RBC) and white blood cells (WBC) were counted manually using Neubauer Haemocytometer (Paul MarienfeldGmbH, Lauda, Koenigshofen, Germany). Hemoglobin concentration (Hb g/dL) was measured spectrophotometrically (Libra S12 biochrom, Cambridge, England) at 540 nm using the cyanomethaemoglobin method (Harikrishnan et al. 2010). Haematocrit (Ht %) was measured by microcentrifuge method (IEC MB centrifuge, Needham, MA, USA) using standard heparinized microhaematocrit capillary tubes (Harikrishnan et al. 2010). In addition, standard routine techniques were used for measuring mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Gholipour –Khani et al. 2017).

**Biochemical parameters**

After centrifugation of blood (3500xg for 5 min), plasma was collected and stored at −18°C until it was analyzed. Glucose, albumin, total protein, triglyceride, cholesterol, LDL and, HDL of plasma were measured by standard spectrophotometric
assays, using Pars Azmoon commercial kits (Gholipour-Khani et al. 2017). Plasma cortisol levels were determined by radioimmunoassay (Grutter and Pankhurst 2000) using Pars Azmoon kit (Gholipour-Khani et al. 2017). Lysozyme activity was measured based on the ability of the lysozyme enzyme to lyse the Micrococcus lysodeikticus bacteria (Kim and Austin 2006).

**ALT, AST, and ALP levels**

Enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured by Pars Azmun commercial kits after plasma separation.

**Serum superoxide dismutase (SOD) activity**

The activity of Serum superoxide dismutase (SOD) was determined spectrophotometrically by the ferricytochrome C method using xanthine/xanthine oxidase as a source of superoxide radicals (Ai et al. 2011).

**Respiratory burst activity (RBA)**

The generation of intracellular superoxide radicals by sole phagocytes was measured by the reduction of nitro-blue tetrazolium (NBT) matching the technique described by and Boesen et al. (2001).

**Alternative complement activity (ACH50)**

Alternative complement activity (ACH50) was measured, according to the method of Tukmechi et al. (2010), using rabbit red blood cells (RRBC) as targets.

**Determination of serum immunoglobulin (Ig) level**

Total immunoglobulin concentration was determined, by following the method described by Siwicki and Anderson (1993), by mixing 50 μL 12% polyethylene glycol (PEG) solution with 50 μL of serum (Sigma, USA). In order to precipitate the immunoglobulin molecules, the mixture was incubated at 25°C for 2 h under constant mixing. After centrifugation at 5000 × g for 15 min, the supernatant was removed and the remaining concentration of protein was measured by subtracting this figure from the total serum protein concentration. Total immunoglobulin concentration was determined using the formula below:

\[
\text{Total Ig (mg mL}^{-1}\text{)} = \text{Total serum protein} - \text{PEG absorbed protein}
\]

**Density challenge**

After six weeks of feeding, fish stock density tripled in each tank (60 fish per tank) and the fish were reared at this density for two weeks. Then, blood samples were taken from these fish by syringe to measure stress parameters including glucose, albumin, total protein, cortisol, and lysozyme according to the methods mentioned above.

**Statistical analysis**

The data obtained from this study were analyzed by one-way analysis of variance (ANOVA) in SPSS software version 16. Significant differences (p < 0.05) among means were determined by Duncan’s multiple range tests. The data were presented as mean ± standard deviation (mean ± SD) in all experimental groups.

**Results**

The comparison of the given data on the weight gain, specific growth rate (SGR), food conversion ratio (FCR), feed intake (g), condition factor (CF), and protein efficiency rate (PER) of the treatment groups are shown in Table 1. The difference in the mean values of SGR, FCR, PER, feed intake, final weight, and weight gain in the treatment groups, compared with the control group, is statistically significant (p < 0.05).

The highest weight gain (21.43 ± 1.15) and specific growth rate (7.29 ± 0.06) were recorded in the treatment group fed with 7 g/kg of lemon verbena and the treatment group fed with 5 g/kg of lemon verbena showed the lowest FCR (1.93) and highest PER (1/43 ± 0.08) (Table 1).

Haematological indices of rainbow trout fed with different levels of lemon verbena extract are shown in Table 2. Some blood indices such as RBC, WBC, Hct, Hb, MCV, MCH, and MCHC are not considerably different between treatments and the control group (p > 0.05). Moreover, no significant difference was observed among treatments and the control group (p >

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**Table 1. Growth parameters of rainbow trout fed with diets containing different levels of lemon verbena extract.**

| Parameters        | Control       | 2.5           | 5             | 7             |
|-------------------|---------------|---------------|---------------|---------------|
| Initial weight (IW) (g) | 28.7 ± 1.78a  | 25.28 ± 2.36a | 25.23 ± 2.18a | 28.38 ± 0.88a |
| Final Weight (FW) (g) | 46.43 ± 0.17ab | 44.76 ± 1.69ab | 46.25 ± 2.26ab | 49.82 ± 1.54b |
| Weight gain (WG) % | 17.73 ± 1.3a  | 19.48 ± 2.4ab  | 21.02 ± 0.48b | 21.43 ± 1.15b |
| FCR               | 2.41 ± 0.15b  | 2.06 ± 0.28ab  | 1.93 ± 0.12ab  | 2.08 ± 0.06a  |
| SGR (%/day)       | 6.84 ± 0.17a  | 7.06 ± 0.25ab  | 7.25 ± 0.05b  | 7.29 ± 0.06b  |
| Feed intake (g)   | 2.41 ± 0.13b  | 2.06 ± 0.28a  | 1.93 ± 0.12a  | 2.08 ± 0.08a  |
| CF                | 1.09 ± 0.1a   | 1.17 ± 0.01a   | 1.16 ± 0.21a  | 1.09 ± 0.08a  |
| PER               | 1.15 ± 0.06a  | 1.35 ± 0.18ab  | 1.43 ± 0.08b  | 1.33 ± 0.05ab  |
| Survival %        | 100a          | 100a           | 100a           | 100a           |

Notes: – Data are expressed as mean ± S.D.
– Values with different superscripts in the same parameter and row are statistically different (p < 0.05).
The density of each treatment triplicated and their amount was no mortality during the density challenge experiment. Cal indices of rainbow trout juveniles after two weeks of being in the control group.

Belonged to the 7 ml/kg group and the lowest amount was between groups. However, the highest amount of ACH50 MCH (pg) 85.47 ± 5.46 86.42 ± 8.8 84.67 ± 10.77 85.26 ± 6.49.

In the present study, RBC, WBC, Hb, Hct, MCV, MCH, and MCHC levels were not significantly different among all groups (p > 0.05). However, experimental treatments had a higher content of IgM in comparison to control. The amount of SOD and RBA in fish, fed with diets containing 7 and 5 g/kg-1 lemon verbena extract, was significantly higher than other treatments (p < 0.05). Moreover, the amount of ACH50 in the 7 g/kg treatment was significantly higher than Control and other experimental treatments (p < 0.05).

Blood biochemical parameters

Table 3 presents the changes in blood biochemical parameters. Plasma glucose levels were not significantly different in the groups fed with different amounts of hydro-alcoholic extract in comparison with the control group (p > 0.05). However, triglyceride, cholesterol, LDL, and HDL levels were remarkably affected by different amounts of the extract in the diets (p < 0.05). Furthermore, the control group demonstrated a higher level of triglyceride, cholesterol, LDL, and HDL compared to the treatments. As shown in Table 2, oral use of the extract in different amounts decreased plasma cortisol (p < 0.05). In addition, Plasma albumin and total protein in groups fed with A. triphylla extract significantly increased along with an increase in plant extract levels (p < 0.05). In other words, the highest levels of plasma albumin and total protein were observed in groups fed with 7 g per kg of the extract. Dietary lemon verbena extracts significantly dropped the plasma lysozyme level (p < 0.05).

Table 3 presents the data on liver enzyme measurements. There was no difference in AST, ALT, and ALP levels among all treatments (p > 0.05).

Immunological indices of rainbow trout juveniles fed on different levels of dietary lemon verbena extract are shown in Table 4. There was a remarkable increase (p < 0.05) in the lysozyme activity, SOD, and Respiratory burst activity (RBA) between treatment compared to the control group but, at the same time (six weeks), according to statistical analysis of data, there were no significant differences (p > 0.05) of ACH50 between groups. However, the highest amount of ACH50 belonged to the 7 ml/kg group and the lowest amount was in the control group.

Changes in some biochemical parameters and immunological indices of rainbow trout juveniles after two weeks of being exposed to density challenge are indicated in Table 5. There was no mortality during the density challenge experiment. The density of each treatment triplicated and their amount was 9.28 kg/m³ for the control group, 8.95, 9.24, 9.96 kg/m³ for 2.5, 5, and 7 g/kg treatments, respectively. According to statistical analysis of data, the levels of plasma glucose and cortisol significantly decreased compared to those of the control group (p < 0.05). However, compared with the control group (p < 0.05), the diets supplemented with plant extract significantly increased the levels of albumin, total protein, and lysozyme. The results showed no significant difference in IgM among all groups (p > 0.05). However, experimental treatments had a higher content of IgM in comparison to control. The amount of SOD and RBA in fish, fed with diets containing 7 and 5 g/kg-1 lemon verbena extract, was significantly higher than other treatments (p < 0.05). Moreover, the amount of ACH50 in the 7 g/kg treatment was significantly higher than Control and other experimental treatments (p < 0.05).

Discussion

The lemon verbena extract had some growth-promoting effects in the present study. The value of the final weight, weight gain, specific growth rate, and protein efficiency rate was significantly higher and the FCR and feed intake were lower in the 7 g/kg treatment, suggesting that the extract may stimulate digestive enzymes and/or gut absorption. Moreover, leaf extract may have negative effects on fish growth performance, as reported in hybrid grouper (Epinephelus lanceolatus×Epinephelus fuscoguttatus♀) and Nile tilapia (Oreochromis niloticus) fed diets supplemented with Ginkgo bilobaand Moringa oleifera leaf extract (Dongmeza et al. 2006; Tan et al. 2018). Such negative effects of the leaf extract on the growth performance of the fish fed with the supplemented diets, were related to lower feed consumption. This may explain no significant effects of dietary oak leaf extract on common carp’s growth rate in a study by Praya et al. (2020). Supporting this hypothesis, Nelumbo nucifera and Psidium guajava leaf extracts improved the growth rate of Mozambique tilapia (Oreochromis mossambicus) and grass carp (Ctenopharyngodon idella) by augmenting feed intake (Gobi et al. 2016; Zhu et al. 2019). These inconsistent results may be related to the kind of extracted species and animal species.

Haematological indices are suitable criteria in reflecting the effects of dietary treatments on meeting the physiological, biochemical, and metabolic needs of animals (Ewuola et al. 2004). In the present study, RBC, WBC, Hb, Hct, MCV, MCH, and MCHC levels were not significantly different among the treatments. Pakravan et al. (2011) reported similar results for Common Carp fed with Willow herb (Epilobium hirsutum) extract. Another study, conducted by Gholipour-Khani et al. (2017), found that RBC, WBC, MCV, MCH, and MCHC of Common Carp were not affected by A. triphylla extract. Although oral

### Table 2: Haematological parameters of rainbow trout fed with diets containing different levels of lemon verbena extract.

| Parameters  | Control          | 2.5  | 5   | 7   |
|------------|------------------|------|-----|-----|
| RBC (10^6/mm³) | 110.47 ± 2.55   | 108.23 ± 2.88 | 111.07 ± 5.29 | 109.7 ± 0.51 |
| WBC (10^3/mm³)  | 1.02 ± 0.02     | 1.11 ± 0.11 | 1.08 ± 0.11 | 1.02 ± 0.02 |
| HB (g/dl)      | 9.43 ± 0.4      | 9.33 ± 0.65 | 9.36 ± 0.77 | 9.26 ± 0.25 |
| HCT (%)        | 26.63 ± 0.47    | 27.26 ± 2.77 | 26.8 ± 2.6 | 24.86 ± 3.5 |
| MCV (µm³)      | 241.1 ± 3.9     | 252.6 ± 34.08 | 242.35 ± 34.10 | 226.69 ± 4.1 |
| MCH (pg)       | 85.47 ± 5.46    | 86.42 ± 8.8  | 84.67 ± 10.77 | 85.26 ± 6.49 |
| MCHC           | 35.88 ± 1.34    | 33.69 ± 4.74 | 36.13 ± 4.69 | 37.48 ± 1.58 |
| Lymphocyte (%) | 46.03 ± 1.65    | 44.76 ± 2.14 | 46.06 ± 3.26 | 45.03 ± 1.36 |
| Monocyte (%)   | 7.16 ± 0.25     | 7.06 ± 0.17  | 7.13 ± 0.3  | 7 ± 0.1    |
| Granulocyte (%)| 46.8 ± 1.86     | 48.16 ± 2.29 | 46.83 ± 3.56 | 47.96 ± 1.34 |

Notes: – Data are expressed as mean ± S.D.
– Values with different superscripts in the same parameter and row are statistically different (p < 0.05).
administering clove basil (Ocimum gratissimum) and ginger (Zingiber officinale) essential oils was useful for improving the growth and immune responses; it had no significant effect on the number of RBC, WBC in tilapia (Oreochromis niloticus) (Brum et al. 2017).

Based on this research, glucose and cortisol changed in fish treated with different dosages of plant extract. In addition, the high levels of plant extract (up to 7 g/kg of diets) decreased cortisol and glucose content. Some nutritional supplements can alter the level of cortisol and glucose in fish plasma, which are two important indicators of the stress response (Nobahar et al. 2014). The level of blood glucose abruptly rises in a short time in order to provide a sufficient amount of energy for fish under stress conditions. Cortisol as a modulator of various physiological processes increases in plasma. Cortisol is considered as the main endpoint of the Hypothalamic-Pituitary-Interrenal (HPI) axis for evaluation of stress response in several fish species like rainbow trout (Barcellos et al. 2011). This reduction reveals that the addition of A. triphylla extract to rainbow trout’s commercial diet does not create stressful situations. In contrast to our result, the study conducted by Binaii et al. (2014) stated that glucose levels did not change with the use of diet, containing nettle extract. This treatment indicates that supplementation with A. triphylla extract changes the plasma volume in treated fish. Currently, few studies reported that using herbal immunostimulants increases the level of serum protein (Hajibegloo and Sudagar 2010). On the contrary, Soltani et al. (2010) and Nya and Austin (2011) found that Garlic and Thyme have no effect on serum proteins levels. Albumin, as the most abundant protein in serum, has an antioxidant role in innate immune response (Harikrishnan et al. 2010). Since the albumin level of treatments increased the level of serum protein (Hajibegloo and Sudagar 2010), according to Immanuel et al. (2009) and Soltani et al. (2010), immunostimulants had no effect on the level of the cholesterol synthesized in the liver transfers to other parts of the body in the form of LDL, but HDL transports the cholesterol from peripheral tissues to the liver. In this respect, the increased excretion of cholesterol through bile (Asgary et al. 2000) may be regarded as the cause of the decrease in the level of cholesterol in the blood of the experimental group fed with lemon verbena extract. Moreover, adding Yarrow (Achillea millefolium) extract to the Rainbow Trout diet has been reported to reduce triglyceride, cholesterol, LDL, and HDL compared with the control group. Furthermore, cholesterol and triglyceride contents decrease in the blood of rainbow trout, catfish, and beluga fed with Silymarin (Silybum marianum) extract (Banaee et al. 2011) and 1% onion powder (Akrami et al. 2015a), respectively.

Serum total protein changes mainly due to alterations in plasmatic volume that is resulted from stressors (Biller et al. 2013). The significant difference in serum protein among the treatments indicates that supplementation with A. triphylla extract changes the plasma volume in treated fish. Similarly, some studies reported that using herbal immunostimulants increases the level of serum protein (Hajibegloo and Sudagar 2010). On the contrary, Soltani et al. (2010) and Nya and Austin (2011) found that Garlic and Thyme have no effects on serum protein levels. Albumin, as the most abundant protein in serum, has an antioxidant role in innate immune response (Harikrishnan et al. 2010). Since the albumin level of treatments increased the level of serum protein (Hajibegloo and Sudagar 2010), according to Immanuel et al. (2009) and Soltani et al. (2010), immunostimulants had no effect on the level of the cholesterol synthesized in the liver transfers to other parts of the body in the form of LDL, but HDL transports the cholesterol from peripheral tissues to the liver. In this respect, the increased excretion of cholesterol through bile (Asgary et al. 2000) may be regarded as the cause of the decrease in the level of cholesterol in the blood of the experimental group fed with lemon verbena extract. Moreover, adding Yarrow (Achillea millefolium) extract to the Rainbow Trout diet has been reported to reduce triglyceride, cholesterol, LDL, and HDL compared with the control group. Furthermore, cholesterol and triglyceride contents decrease in the blood of rainbow trout, catfish, and beluga fed with Silymarin (Silybum marianum) extract (Banaee et al. 2011) and 1% onion powder (Akrami et al. 2015a), respectively.

Table 3. Some of the blood biochemical parameters of rainbow trout fed with diets containing different levels of lemon verbena extract.

| Parameters          | Control          | 2.5             | 5              | 7              |
|---------------------|------------------|-----------------|----------------|----------------|
| Glucose (mg/dl)     | 43.47 ± 3.6      | 41.66 ± 2.88    | 37.33 ± 2.51   | 37.3 ± 3.78    |
| Triglyceride (mg/dl)| 1109.7 ± 15.04   | 1027.7 ± 42.00  | 1001.01 ± 28.16 | 986.67 ± 15.27 |
| Cholesterol (mg/dl)| 475.67 ± 4.58    | 459 ± 23.06     | 401.63 ± 7.63  | 392 ± 15.93    |
| LDL (mg/dl)         | 41.33 ± 1.15     | 41.2 ± 2.35     | 40.86 ± 1.8    | 36.20 ± 3.15   |
| HDL (mg/dl)         | 95.36 ± 6.25     | 86.63 ± 9.16    | 85.93 ± 14.56  | 76.23 ± 0.49   |
| Cortisol (mg/dl)    | 17.1 ± 1.66      | 12.93 ± 1.64    | 10.4 ± 1.15    | 10.13 ± 0.89   |
| Albumin (mg/dl)     | 1.47 ± 0.06      | 1.64 ± 0.03     | 1.6 ± 0.09     | 1.84 ± 0.14    |
| Total Protein (g/dl)| 3.66 ± 0.25      | 3.8 ± 0.2       | 3.9 ± 0.17     | 4.1 ± 0.1     |
| ALT (U/L)           | 178.21 ± 73.71   | 322.14 ± 14.34  | 338.7 ± 67.09  | 330.16 ± 33.83 |
| AST (U/L)           | 17.1 ± 46.23     | 16 ± 5.34       | 16 ± 83.75     | 17.1 ± 76.44   |
| ALP (µU/mL)         | 628.3 ± 67.03    | 631.63 ± 33.61  | 636.34 ± 67.29 | 636.34 ± 67.29 |

Notes: – Data are expressed as mean ± S.D. 
– Values with different superscripts in the same parameter and row are statistically different (p < 0.05).

Table 4. Some of the immunological indices of rainbow trout fed with diets containing different levels of lemon verbena extract.

| Parameters          | Control          | 2.5             | 5              | 7              |
|---------------------|------------------|-----------------|----------------|----------------|
| Lysozyme (IU/m)     | 23.66 ± 2.51     | 24 ± 1*         | 25.66 ± 3.21   | 29 ± 1b        |
| IgM (mg/l)          | 30.7 ± 2.15      | 33.7 ± 1.97     | 35.2 ± 2.08    | 39.84 ± 1.71   |
| SOD (unit/ml)       | 28.3 ± 1.78      | 27.5 ± 1.63     | 31.3 ± 0.98    | 33.29 ± 2.84   |
| RBA (O.D)           | 981.8 ± 21.4     | 1012 ± 32.16    | 1120 ± 31.48   | 1098 ± 22.16   |
| ACHS05 (unit/ml)    | 83.8 ± 9.07*     | 99.16 ± 11.43   | 108.14 ± 3.52  | 116.12 ± 6.43  |

Notes: – Data are expressed as mean ± S.D. 
– Values with different superscripts in the same parameter and row are statistically different (p < 0.05).
albumin. These differences may depend on environmental factors such as temperature, pH, and salinity, which influence lipid content in fish (Citarasu 2010). In addition, different climates, ages, the weight of samples, and plant compounds as well as the duration of the experiments can influence responses (Citarasu 2010). Lysozyme is a group of enzymes with antibacterial activity, which is characterized by the ability to damage the bacterial cell wall (Ahmadi et al. 2012). Lysozyme content in plasma increased significantly in groups fed with plant extract. Some researchers demonstrated that herbal extracts promote serum lysozyme activity in fish, which is congruent with the result of this study (Zhang et al. 2009).

In the present study, no significant difference was observed in the activity of alanine, ALP, AST, and ALP enzymes among all treatments, indicating that oral administration of A. Triphylia hydroalcoholic extract did not damage the liver tissue. The results are consistent with studies done by Gholipour-Khani et al. (2017), Zeppenfeld et al. (2017), and Gholipour-Khani et al. (2017). Damage to cell membranes in various tissues such as liver, heart, muscle, kidney, pancreas, spleen, erythrocytes, and gills may release AST and ALT, found in mitochondria, into the bloodstream. As this process continues, their activity in blood increases (Banaee et al. 2011). Soltan and El-Laithy (2008) demonstrated that there were no considerable differences in respiratory burst activity among treatments; however, the group tested with 2% L. edodes mushroom extract had higher respiratory burst.

The SOD is a metallo-enzyme that plays major roles in the protection of cells against oxidative damage (Metaxa et al. 2006). In the present study, all experimental treatments had a higher content of SOD in comparison to control. However, results of before and after density challenge showed that the amount of SOD in T2 and T3 treatments were significantly higher than control ($p<0.05$). Contrary to this study, Yuan et al. (2007) noticed that there was no significant difference in SOD activity between the 0.5% and 1% herbal immunity stimulants such as herbal derivatives (Christybapita et al. 2007). Similar to the present study, Jahanjoo et al. (2018) reported the increased Ig level in beluga after feeding with nettle for six weeks. In the results of ACH50, all experimental treatments, except 2.5 g/kg after density challenge, had a higher amount of ACH50 in comparison to control, which may help to identify and eliminate bacterial agents by phagocytosis (Jahanjoo et al. 2018). Several researchers reported an increase in complement activity, following the administration of different immunostimulants such as herbal derivatives. The results showed that the RBA level of all experimental treatments was higher than the control group. Moreover, the difference between 5 and 7 g/kg treatments and other groups was significant ($p<0.05$). In the study of Binaii et al. (2014) and Akrami et al. (2015a), greater respiratory burst activity was reported in beluga administered with dietary nettle and onion after feeding for eight weeks, respectively. These findings are in contrast to the results of the investigation of Chitsaz et al. (2018), which demonstrated that there were no considerable differences in respiratory burst activity among treatments; however, the group tested with 2% L. edodes mushroom extract had higher respiratory burst.

The fish cultured in intensive systems are continuously exposed to stress, which leads to significant biochemical and physiological changes. In our investigation, the highest level of cortisol and glucose were observed in the control group after the stocking density challenge. Changes in the level of plasma cortisol and glucose reflect their concerted actions on energy metabolism. Similarly, Bianca (2009) suggested that the level of cortisol quickly increases after exposure to acute stress and nonstandard conditions are restored in few hours. In spite of the fact that cortisol may be affected by species, feeding, reproductive cycles, seasonal cycles, photoperiod, husbandry condition, and sampling, it is widely used as a long-term and short-term stress condition

### Table 5. Changes in some biochemical parameters and immunological indices of rainbow trout after 2 weeks of exposure to the density challenge.

| Parameters          | Control   | 2.5       | 5         | 7         |
|---------------------|-----------|-----------|-----------|-----------|
| Glucose (mg/dl)     | 57.66 ± 4.93$^a$ | 48.00 ± 2.64$^b$ | 44.03 ± 1.00$^{ab}$ | 39.33 ± 2.51$^b$ |
| Albumin (g/dl)      | 1.48 ± 0.09$^a$   | 1.64 ± 0.02$^b$   | 1.71 ± 0.08$^b$   | 2.03 ± 0.06$^b$   |
| Total Protein (g/dl)| 4.2 ± 0.3$^a$     | 4.56 ± 0.25$^b$   | 5.1 ± 0.3$^b$     | 5.83 ± 0.25$^b$   |
| Cortisol (µg/dl)    | 41.3 ± 1.05$^a$   | 29.63 ± 0.55$^b$  | 23.66 ± 0.15$^b$  | 22.2 ± 1.21$^b$   |
| Lysozyme (IU/m)     | 14 ± 2$^a$       | 27.66 ± 0.57$^b$  | 22 ± 2$^b$       | 28.66 ± 2.51$^c$  |
| IgM (mg/l)          | 19.4 ± 2.02$^a$  | 21.7 ± 1.14$^a$   | 20.2 ± 2.24$^a$   | 21.04 ± 1.71$^a$  |
| SOD (unit/ml)       | 16.4 ± 1.42$^a$  | 21.5 ± 1.73$^a$   | 25.4 ± 1.14$^b$   | 27.12 ± 2.14$^b$  |
| RBA                 | 970.8 ± 32.1$^a$ | 992.3 ± 17.16$^b$ | 1020 ± 31.48$^b$ | 1112.2 ± 21.16$^b$ |
| ACH50 (unit/ml)     | 105.8 ± 7.71$^a$ | 86.16 ± 11.43$^a$ | 107.14 ± 3.12$^a$ | 121.12 ± 9.13$^b$ |

Notes: - Data are expressed as mean ± S.D.

- Values with different superscripts in the same parameter and row are statistically different ($p<0.05$).
index. In addition, different kinds of teleost fish lack aldosterone and it seems that mineral regulatory processes occur under dominant cortisol conditions (Stolte et al. 2008). The present study revealed that plasma glucose levels significantly decreased from E1 to E3 treatments. It can be associated with the increased level of catecholamines and cortisol because they are considered principal hormones in controlling carbohydrate metabolism. These results are consistent with those obtained by Pickering et al. (1982) who proved that stress may increase the secretion of catecholamines, which initially suppressed insulin secretion and subsequently increased the plasma level of glucose (Wright et al. 2000). Since Rainbow Trout does not consume large quantities of glucose in the wild, it is not surprising that they are involved in a mechanism that transfers glucose rapidly from the bloodstream into muscle and fat (Wright et al. 2000).

The level of albumin, total protein, lysozyme, SOD, ACH50, and RBA in the blood of fish, fed with Lemon verbenàs extract and exposed to stocking density challenge, were significantly higher than those of the control group. The IgM levels increased along with increasing doses of plant extract; however, there was no significant increase. Our findings showed that Lemon verbenàs extract supplemented diet improved the immunological indices in experimental groups compared to the control group. It also reported that the level of lysozyme in African Catfish (Clarias gariepinus) increased after the stocking density challenge (Wang et al. 2013). On the contrary, Mauri et al. (2011) reported that there was no significant change in lysozyme activity of European sea bass (Sparus aurata) under the challenge of high density for 15 days. Furthermore, there are some other findings, showing an increase in serum lysozyme activity of rainbow trout fingerlings fed and treated with Quercus brantii extract (Bohlouli et al. 2016) and supplemented with Aloe vera extract (Haghighi et al. 2014). ACH50 increased in experimental groups supplemented with A. triphylla extract especially in E3. Similar to these findings, some data was reported in rainbow trout fingerlings supplemented with Persian oak (Bohlouli et al. 2016) and traditional Korean herbs supplemented with Paralichthys olivaceus (Harikrishnan et al. 2012).

Dietary supplement of A. triphylla extract may be a functional food additive because it was found to suppress stress and augment fish antioxidant and immune functions under normal and stressful conditions. Therefore, the addition of A. triphylla extracts up to 7 gr/Kg of diet is recommended for Rainbow Trout since it has no harmful effects on different tested factors. Thus, it is able to modulate some immune parameters in rainbow trout.

**Ethical approval**

In this study, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All conditions (stocking density, water parameters, and so on) were designed according to the welfare-related assessments, suitable for O. mykiss. The trials with the rainbow trout were approved by Islamic Azad University of Azadshahr’s Local Ethical Committee for Animal Studies (approved on 14/10/2019, Acceptance No:9).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**References**

Aanyu M. 2016. Effects of phytopharmacological admixture on growth and nutritional physiology of Nile tilapia (Oreochromis niloticus) [Ph. D thesis]. Scotland: University of Stirling. p. 110.

Acar Ü, Parrino V, Kesiçi OS, Lo Paro G, Saoca C, Abbate A, Yilmaz S, Fazio F. 2018. Effects of dietary levels of pomegranate seed oil on some blood parameters and disease resistance against Yersinia ruckeri in rainbow trout. Front. Physiol. 9:1956.

Ahmadi K, Banaee M, Vosoghei AR, Mirvaghefei AR, Bet al A. 2012. Evaluation of the immunomodulatory effects of silymarin extract (Silybum marianum) on some immune parameters of rainbow trout (Oncorhynchus mykiss). Acta Ichthyologica et Piscatoria. 42(2):113–120.

Ahmed I. 2020. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquacult Int. 28:869–899.

Ai Q, Xu H, Mai K, Xu W, Wang J, Zhang W. 2011. Effects of dietary supplementation of Bacillus subtilis and fructo-oligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, Larimichthys crocea. Aquaculture. 317(1–4):155–161. doi:10.1016/j.aquaculture.2011.04.036.

Akrami R, Gharaei A, Mansour MR, Galeshi A. 2015a. Effects of dietary onion (Allium cepa) powder on growth, innate immune response and hematobiological parameters of beluga (Huso huso Linnaeus, 1754) juvenile. Fish Shellfish Immunol. 45(2):828–834. doi:10.1016/j.fsi.2015.06005.a.

Akrami R, Nasri-Tajan M, Jahedi M, Razeghi Mansour MR. 2015b. Effects of dietary synbiotic on growth, survival, lactobacillus bacterial, blood indices and immunity of Beluga (Huso huso Linnaeus, 1754) juvenile. Aquaculture. 420:121–129. doi:10.1016/j.aquaculture.2011.04.055.

Ahmadi K, Banaee M, Vosoghei AR, Mirvaghefei AR, Bet al A. 2012. Effects of dietary supplementation of Bacillus subtilis and fructo-oligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, Larimichthys crocea. Aquaculture. 317(1–4):155–161. doi:10.1016/j.aquaculture.2011.04.036.

Akrami R, Gharaei A, Mansour MR, Galeshi A. 2015a. Effects of dietary onion (Allium cepa) powder on growth, innate immune response and hematobiological parameters of beluga (Oncorhynchus mykiss) and its preventive efficacy against Yersinia ruckeri infection. Iran J Fish Sci. 19(3):1304–1318.

Babaealian A, Azari Takami G, Afsharnasab M, Zargar A. 2020. Effects of commercial herbal oil mixture on some hematological, biochemical and immunological parameters of rainbow trout (Oncorhynchus mykiss) and its preventive efficacy against Yersinia ruckeri infection. Iran J Fish Sci. 19(3):1304–1318.

Banaee M, Sureda A, Mirvaghefei AR, Rafei GR. 2011. Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (Oncorhynchus mykiss). Fish Physiol Biochem. 37(4):887–896. doi:10.1007/s10695-011-9486-2.

Barcellos LJJ, Volpato G, Luiz B, Coldebelia I, Ferreira D. 2011. Chemical communication of handling stress in fish. Physiol Behav. 103(3–4):372–375. doi:10.1016/j.physbeh.2011.03.009.

Bhadauria M. 2012. Propolis prevents hepato-renal injury induced by chronic exposure to carbon tetrachloride. Evid-Based Complement Altern Med. 1–12. doi:10.1155/2012/235358.

Bianca MP. 2009. Farmed fish welfare-suffering assessment and impact on product quality. Ital J Anim Sci. 8(supl):1:139–160. doi:10.4081/ijas.2009.s1.139.

Billot J, Takahashi L, Pilarski F, Sebastião FA, Urbinati EC. 2013. Serum bactericidal activity as indicator of innate immunity in pacu Piaractus mesopotamicus (Holmberg, 1887). Arquivo Bras Med Vet Zoot. 65(6):1745–1751. doi:10.1590/0020-0935201300600023.

Binaí M, Ghiasi M, Farabi SMV, Pourgholam R, Fazli H, Safari R, Alavi SE, Taghavi MJ, Bankehsaz Z, et al. 2014. Biochemical and hemato-
immunological parameters in juvenile beluga (Huso huso) following the supplemental treatment with nettle (urtica dioica). Fish Shellfish Immunol. 36 (1):46–51. doi:10.1016/j.fsi.2013.10.001.
Boesen HT, Larsen MH, Larsen LH, Ellis AE. 2001. In vitro interactions between rainbow trout (Oncorhyncus mykiss) macrophages and Vibrio anguillarum serogroup O2a. Fish Shellfish Immunol. 11(5):415–431. doi:10.1006/fsim.2000.0328.
Bohlouli S, Ghaedi G, Heydari M, Rahmani A, Sadeghi E. 2016. Effect of dietary Persian oregan (Quercus brantii var.persica) fruit extract on survival growth performance, hematological and immunological parameters in rainbow trout, Oncorhyncus mykiss, fingerlings. Aquac Nutr. 22 (4):745–751. doi:10.1111/anu.12290.
Brum A, Pereira SA, Owatari MS, Chagas EC, Chaves FCM, Mouriño JLP, Chitsaz H, Akrami R, Ahmadi Z. 2018. Effect of dietary essential oils of clove basil and ginger on Nile tilapia (Oreochromis niloticus) following challenge with Streptococcus agalactiae. Aquaculture. 486(1):235–243. doi:10.1016/j.aquaculture.2016.10.040.
Chitsaz H, Akrami R, Ahmadi Z. 2018. Effects of mushroom (Lentinula edodes) extract on growth performance, immune response and hemato-biochemical parameters of great sturgeon juvenile (Huso huso Linnaeus, 1754). Iran J Aquat Anim Health. 4(1):29–48. http://ijahj.ir/article-1-164-en.html.
Christybabita D, Divyaganesanwari M, Michael RD. 2007. Oral administration of Eclipta alba leaf aqueous extract enhances the non-specific immune responses and disease resistance of Oreochromis mossambicus. Fish Shellfish Immunol. 23(4):840–852. doi:10.1016/j.fsi.2007.03.010.
Citarsu T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquac Int. 18(3):403–414. doi:10.1007/s10499-009-9253-7.
Cristea V, Antache A, Grecu I, Docan A, Dediu L, Mocanu MC. 2012. The use of phytobiotics in aquaculture. Univ Agric Sci Vet. 57:250–255.
DirekBurasarakom S. 2004. Application of medicinal herbs to aquaculture in Asia. Walaikul J Sci Technol. 1(1):7–14.
Dongmeza E, Siddhurajupa F, Francis G, Becker K. 2006. Effects of dehydrated methanol extracts of moringa (Moringa oleifera Lam) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (Oreochromis niloticus (L.)). Aquaculture. 261:407–422.
Ewuala EO, Foyaya OA, Gbore FA, Adebunmi AI, Akanji RA, Ogunlade JT, Adeneye JA. 2004. Physiological response of growing west African dwarf goats fed groundnut shell-based diets as the concentrate supplements. Bowen J Agric. 1(1):61–69. doi:10.4134/bja.v11i1.41855.
Fazio F, Ferrantelli V, Piccione G, Saoca C. 2018. Biochemical and hemato-logical parameters in European sea bass (Dicentrarchus labrax Linnaeus, 1758) and Gilthead sea bream (Sparus aurataLinnaeus, 1758) in relation to temperature. Vet Arhiv. 88:397–411.
Fazio F. 2019. Fish hematolgy analysis as an important tool of aquaculture: a review. Aquaculture. 500:237–242.
Gholipour -Khani H, Jamali F, Jafaryan H, Gholamalipour Alamdari E.2017. Changes in complement responses in Gilthead seabream (Sparus aurata) and European seabass (Dicentrarchus labrax) under crowding stress, plus viral and bacterial challenges. Fish Shellfish Immunol. 30(1):182–188. doi:10.1016/j.fsi.2010.01.006.
Metaxa E, Deviller G, Pagand P, Alliaume C, Casellas C, Blancheton JP. 2006. High rate algal pond treatment for water reuse in a marine fish recirculation system: water purification and fish health. Aquaculture. 252(1):92–101. doi:10.1016/j.aquaculture.2005.11.053.
Nobahar Z, Gholipour-Kanani H, Kookooli SH, Jafarian H. 2014. Assessment of stress response in great sturgeon (Huso huso) associated with dietary intake of some herbal plants. Iran J Aquat Ani Health. 1(1):63–69.
Nya EJ, Austin B. 2011. Development of immunity in rainbow trout (Oncorhynchus mykiss) induced by probiotics. Fish Shellfish Immunol. 21(5):513–524. doi:10.1016/j.fsi.2006.02.007.
Mauri I, Romero A, Acretote J, Mac-Kenzie S, Roher N, Callol A, … Tort L. 2011. Changes in complement responses in Gilthead seabream (Sparus aurata) and European seabass (Dicentrachus labrax) following crowding stress, plus viral and bacterial challenges. Fish Shellfish Immunol. 30(1):182–188. doi:10.1016/j.fsi.2010.01.006.
Pakravan S, Haimoradliooo L, Ghorbani R. 2011. Effect of dietary willow herb, Epilobium hirsutum extract on growth performance, body composition, haematological parameters and Aeromonas hydrophila challenge on common carp, Cyprinus carpio. Aquaculture. 43(6):861–891. doi:10.1111/1369-7057.12011.x.
Paraya BA, Hoseini SM, Hoseinifar SH, Van Doand H.2020.E. Effect of dietary essential oils of clove basil and Aloe vera extract supplemented feed on hematological and biological parameters of Cyprinus carpio Linnaeus, 1758. Iran J Aquat Anim Health. 4(1):61–10. doi:10.18696/acpubj.ijah.3.1.1.
Gobi N, Ramya C, Vasueharan B, Malaikozhundan B, Vijayakumar S, Murugan K, Benelli G. 2016. Oreochromis mossambicus diet supplementation with Psidium guajava leaf extracts enhance growth, immune, anti-oxidant response and resistance to Aeromonas hydrophila. Fish Shellfish Immunol. 58:572–583.
Grutter AS, Pankhurst NW. 2000. The effects of capture, handling, confine-ment and ectoparasite load plasma levels of cortisol, glucose and lactate in the coral reef fish Hemigymnus melapterus. J Fish Biol. 57(2):391–401. doi:10.1046/j.1095-8649.2000.t02179.x.
Haghhighi M, Sharif Rohani M, Samadi M, Tavoli M, Eslami M, Yusefi R. 2014. Study of effects Aloe vera extract supplemented feeding on hematological and immunological indices of rainbow trout (Oncorhynchus mykiss). Int J Adv Biol Biomed Res. 2(6):2143–2154. Available online at http://www.ijaabbr.com.
Hajibegllo A, Sudagar M. 2010. Immune response of common carp (Cyprinus carpio) fed with herbal immunostimulants diets. J Anim Vet Adv Agric J. 59(13):1631839–1184772. http://www.medwelljournals.com/fullte...
Soltani MA, El-Laithy SM. 2008. Evaluation of fermented silage made from fish, tomato and potato by-products as a feed ingredient for Nile tilapia, Oreochromis niloticus. Egypt J Aquat Biol Fish. 12:25–41. doi:10.21608/ejabf.2008.1969.

Soltani M, Sheikhzadeh N, Ebrahimzadeh-Mousavi H, Zargar A. 2010. Effects of Zataria multiflora essential oil on innate immune responses of common carp (Cyprinus carpio). J Fish Aquat Sci. 5(4):191–199. http://www.academicjournalsinc.com.

Stolte EH, De Mazon Aurelia F, Leon-Koosterziel KM, Jesiak M, Bury N, Sturm A, Flik G, Karen M. 2008. Corticosteroid receptors involved in stress regulation in common carp, Cyprinus carpio. J Endocrinol. 198:403–417. doi:10.1677/JOE-08-0100.

Sudagar M, Hajibegloo A. 2010. Effect of plant extracts supplemented diets on immunity and resistance to Aeromonas hydrophila in common carp Cyprinus carpio. Res Agric J Anim Sci. 5(2):119–12726-34. http://docsdrive.com/__J/119-127.pdf.

Taheri Mirghaed A, Hoseini SM, Ghelichpour M. 2018. Effects of dietary 1,8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbowtrout (Oncorhynchus mykiss), Fish Shellfish Immunol. 81:182–188.

Tan X, Sun Z, Liu Q, Ye H, Zou C, Ye C, Wang A, Lin H. 2018. Effects of dietary ginkgo biloba leaf extract on growth performance, plasma biochemical parameters, fish composition, immune responses, liver histology, and immune and apoptosis-related genes expression of hybrid grouper (Epinephelus lanceolatus♀ × Epinephelus fuscoguttatus♂) fed high lipid diets. Fish Shellfish Immunol. 72:399–409.

Tukmechi A, Owaghi A, Mohebbat A. 2010. In vitro antibacterial activities of ethanol extract of Iranian propolis (EEIP) against fish pathogenic bacteria (Aeromonas hydrophila, Yersinia ruckeri and Streptococcus iniae). Braz. J Microbiol. 41(4):1086–1092. doi:10.1590/S1517-83822010000400030.

Wang X, Dai W, Xu M. 2013. Effects of stocking density on growth, non-specific immune response, and antioxidant status in African Catfish (Clarias gariepinus). The Israeli Journal of Aquaculture. 65:830–836.

Wright JR, Bonen A, Michael Conlon J, Pohajdak B. 2000. Glucose homoeostasis in the teleost fish tilapia: insights from Brockmann body xenotransplantation studies. Am Zool. 40(2):234–245. doi:10.1093/icb/40.2.234.

Yamamoto Y, Oue E. 2006. Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. Biosci, Biotechnol, Biochem. 70(4):933–939. doi:10.1271/bbb.70.933.

Yuan C, Li D, Chen W, Sun F, Wu G, Gong Y, Han X. 2007. Administration of herbal immunoregulation mixture enhances some immune parameters in carp (Cyprinus carpio). Fish Physiol Biochem. 33(2):93–101. doi:10.1007/s10695-006-9120-7.

Zargar A, Rahimi-Afzal Z, Soltani M, Taheri Mirghaed A, Ebrahimzade Mosavi HA, Soltani M, Yousef P. 2019. Growth performance, immune response and disease resistance of rainbow trout (Oncorhynchus mykiss) fed Thymus vulgaris essential oils. Aquac Res. 2019(50):3097–3106.

Zeppenfeld CC, Hernández DR, Santinón JJ, Heinzmann BM, Da Cunha MA, Schmidt D, Baldisserotto B. 2016. Essential oil of Aloysia triphylla as feed additive promotes growth of silver catfish (Rhamdia quelen). Aquac Nutr. 22(4):933–940. doi:10.1111/anu.12311.

Zeppenfeld CC, Saccol EMH, Pês TS, Salbego J, Koakoski G, Dos Santos AC, Caron BO. 2017. Aloysia triphylla essential oil as food additive for Rhamdia quelen–stress and antioxidant parameters. Aquac Nutr. 23(6):1362–1367. doi:10.1111/anu.12511.

Zeppenfeld CC, Toni C, Becker AG, dos Santos Miron D, Parodi TV, Heinzmann BM, Da Cunha MA. 2014. Physiological and biochemical responses of silver catfish, Rhamdia quelen, after transport in water with essential oil of Aloysia triphylla. Aquaculture. 418-419:107–107. doi:10.1016/j.aquaculture.2013.10.013.

Zhang G, Gong S, Yu D, Yuan H. 2009. Propolis and Herba epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, Myxocyprinus asiaticus. Fish Shellfish Immunol. 26(3):467–472. doi:10.1016/j.fsi.2009.01.011.

Zhu Y, Hu P, Yao J, Xu D, Xu Y, Tan Q. 2019. Optimal dietary alcoholic extract of lotus leaf improved growth performance and health status of grass carp (Ctenopharyngodon idellus). Fish Shellfish Immunol. 93:1–7.