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RESEARCH ARTICLE

Preliminary assessment of the effect of dietary *Pelargonium sidoides* extract on the haematological profile of common carp, *Cyprinus carpio* Linneaus, 1758

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Abstract

A preliminary study was conducted to evaluate the effect of the *Pelargonium sidoides* extract on the haematological profile of common carp, *Cyprinus carpio*. The fish (mean body weight 0.67±0.05 g) were fed with experimental diets prepared by using the supplementation of *P. sidoides* extract (0, 1, 3 and 5 ml 100 g^{-1}) for 90 days. The highest hemoglobin concentration was registered in 3 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (10.50±0.45 g l^{-1}) and 5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (11.02±0.20 g l^{-1}). Among the *P. sidoides* extract-supplemented groups, the highest white blood cell values were also found in 5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (28.98±1.62 G l^{-1}). The highest values of mean corpuscular hemoglobin concentration ($P < 0.05$) were detected in 5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet. Red blood cell, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin levels were not affected by any of the experimental diets ($P>0.05$). The results of the present work have demonstrated that *P. sidoides* extract-based diet affect basic haematological profile of common carp.

Keywords: Common carp, *Cyprinus carpio*, *Pelargonium sidoides*, haematological profile

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Introduction

For centuries, medicinal plants have had primitive and helpful roles for humanity. Herbal medicine is a developing field of alternative medicines at the present time. Many active ingredients in manufactured drugs are derived from plant compounds and these plants have a wide range of use (Pakravan *et al*. 2012). Plants and plant extracts are safer than chemical products, thus natural products are becoming more popular, since drugs of synthetic origin may have a

negative impact on the environment and parasite resistance to poisonous chemicals can develop after repeated applications (Magi and Sahk 2003; Banaee *et al.* 2017). Medicinal plants have various effects such as antistress, growth enhancement, appetite promotion, immunostimulation, aphrodisiac and antimicrobial stuffs due to the active principles such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids and essential oils (Kumar Bairwa *et al.* 2012; Ghadikolaei *et al.* 2017). In recent years, the use of these medical plants has increased in fish culture (Turan *et al.* 2007; Banaee *et al.* 2017; Ahmadi *et al.* 2012; Mohammadi *et al.* 2018).

Pelargonium sidoides extract is one of the most important medicine herbs used in folk medicine by the Southern African native people. *Pelargonium* containing phytopharmaceuticals, elaborated from the traditional medicine, is nowadays successfully employed in modern phytotherapy in Europe (Haidvogl *et al.* 1996). Polymeric polyphenols and coumarins have been identified as the principal ingredients (Koch and Iber 2003). Most of the coumarins contain a methoxy function; functionality that is responsible for their antibacterial activity. Gallic acid and its methyl ester are present in large amount. These were identified as the prominent immunomodulatory principle for this herbal medicine (Kayser and Kolodziej 1998). *P. sidoides* is also rich in photochemical, vitamins, minerals and amino acids that enhance the body's functioning and protect it against diseases (Kolodziej *et al.* 2003). Some studies have been done in which herbs, as dietary additives, were fed to fish and crayfish. The focus of these studies includes their use as feeding attractants and their effects on growth, survival and immune system activity (Ji *et al.* 2007; Lee *et al.* 2004; Kwon *et al.* 1999). Besides, Turan *et al.* (2011; 2012) reported that *Pelargonium sidoides* extract is useful to improve growth, feed utilization and survival rate in common carp and freshwater crayfish, juvenile diets.

More recently, dietary supplementation of phytobiotic in common carp aquaculture has been reviewed by Dawood and Koshio (2016). Common carp (*Cyprinus carpio*) is an economical important aquaculture species worldwide (FAO 2014) which account for 71.9% of freshwater production (Dawood and Koshio 2016). However, to the best of our knowledge, there is a lack of data on the use of *P. sidoides* extract in diets for fish hematology. In the present study we aimed to understand whether *P. sidoides* extract included in the diet affected the haematological parameters of the common carp.

Materials and Methods

Fish and rearing conditions

The common carp, *C. carpio*, with mean body weight 0.67±0.05 g were fed with a commercial carp diet, and stocked into 100 L aquaria at a density of 15 fish per aquarium. Experiment was conducted in aquaria (n=12) with the dimensions of 0.8x0.4x0.4 m (length x width x height). The aquaria were equipped with

aeration and supplied with continuously flowing water (2Lmin^{-1}), and controlled temperature (24±1°C). The photoperiod was maintained on a 12-h light: 12-h dark schedule. The average water temperature was 24±1ºC, and the oxygen content of the water was 5.76±0.35 ppm. At pH 7.9±0.3, the ammonia nitrogen content did not exceed 0.1 mg N-NH_4/l, and nitrite nitrogen was no greater than 0.04 mg N-NO_2/l.

Experimental procedures

Fish were fed with their respective diet to ca. 10% body weight day-1 twice daily with a pelleted feed. Fish were fed diets containing four doses of *P. sidoides* extract (0; 1; 3 and 5%) for 90 days in June-August 2018. Carp diets (Pınar Co. Turkey, 45% protein, 12% lipid (on wet basis)) were used to prepare experimental diets. *P. sidoides* extract (UMCA®) was supplied by Dr. Willmar Schwabe GmbH&Co. (Ettlingen, Germany). At the end of the experimental period, fish from each aquaria were anesthetized with MS-222 (same dose as used for fish allocation) and weighed individually. Five fish from each aquaria were randomly selected for hematological parameter analysis.

Haematological analyses

At the end of the experiment, about 1 mL of blood was taken from fish using the heart puncture technique. Blood from common carp was collected in coded 1.5 mL heparinized plastic tubes, stored on ice and centrifuged within 30 min of their collection. The blood samples were analyzed at the Biochemistry Laboratory of Iskenderun Maternity and Children's Hospital, Turkey, within 2 h of collection. Hematological parameters for blood samples analysis were red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). These values were determined using an automated hematology analyzer (Abbott Cell-Dyn 3700, USA). The resultant value is the number of erythrocytes in T l^{-1} (T – tera = 10^{12}) and number of leukocytes in G l^{-1} (G – giga = 10^9). Haemoglobin content was expressed in g per litre. The haematocrit value was measured in micro-heparinized capillaries, and is given %. The value of the mean corpuscular volume (MCV) was expressed in femtolitres (fl). The mean corpuscular haemoglobin (MCH) value was given in picograms (pg). The mean corpuscular haemoglobin concentration (MCHC) was calculated as a quotient of Hb and HCT and expressed in g l^{-1}.

Statistical analysis

Results were presented as the average (±standard error) and were subjected to a one-way analysis of variance to determine if there was difference among treatments. Duncan test was used to compare the means of the treatments when differences occurred (Norusis 1993). Significance of difference between experimental groups was determined at a significance level of $P<0.05$.

Results

Mean hematological parameters of common carp at the end of the experimental period under the four treatment combinations are given in Table 1.

The highest haemoglobin concentration was registered in 3 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (10.50±0.45 g l^{-1}) and 5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (11.02±0.20 g l^{-1}). These values were significantly higher ($P < 0.01$) than those registered in 1 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (9.26±0.29 g l^{-1}) and control groups (8.63±0.48 g l^{-1}).

Table 1. Hematological parameters of Common carp, *Cyprinus carpio* fed with diets containing *Pelargonium sidoides* extract for 90 days*

***P. sidoides* extract (ml 100 g^{-1})**	**Control**	**1**	**3**	**5**
Final fish weight (g)	9.32±0.68^{a}	9.80±0.47^{a}	12.10±0.75^{b}	14.34±0.41^{c}
RBC (T l^{-1})	1.19±0.12^{a}	1.37±0.06^{a}	1.43±0.08^{a}	1.50±0.09^{a}
WBC (G l^{-1})	21.73±0.75^{a}	23.10±0.45^{a}	23.76±0.55^{a}	28.98±1.62^{b}
Hb (g l^{-1})	8.63±0.48^{a}	9.26±0.29^{a}	10.50±0.45^{b}	11.02±0.20^{b}
HCT (%)	35±1.73^{a}	36±2.08^{a}	37±1.53^{a}	36±1.45^{a}
MCV (fl)	298.26±29.67^{a}	264.15±21.72^{a}	257.94±6.43^{a}	245.75±7.71^{a}
MCH (pg)	73.21±5.32^{a}	68.03±4.48^{a}	73.18±0.83^{a}	74.02±3.32^{a}
MCHC (g l^{-1})	24.67±0.62^{a}	25.99±2.31^{ab}	28.40±0.78^{ab}	30.11±0.87^{b}

*Values (mean ± S.E. of triplicate) with different superscripts in each column indicate significant differences ($P<0.05$). HCT– haematocrit value, RBC – number of red blood cells, Hb – haemoglobin concentration, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, WBC – numbers of leukocytes.

The highest values of MCHC were detected in 5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (30.11±0.87 g l^{-1}) compared to those in control (24.67±0.62 g l^{-1}), 1 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (25.99±2.31 g l^{-1}) and 3 ml 100 g^{-1} *P. sidoides* extract-supplemented diet (28.40±0.78 g l^{-1}) ($P < 0.05$) (Table 1). In the end of experiment, RBC, HCT, MCV and MCH levels were not affected by any of the experimental diets ($P>0.05$).

The final weight was significantly increased in carps fed with *P. sidoides* extract-supplemented diets in comparison with the control groups ($P<0.05$). Among the *P. sidoides* extract-supplemented groups, the fish fed with 5 ml 100 g^{-1} *P. sidoides* extract exhibited significantly more weight than fish fed with 1 and 3 ml 100 g^{-1} *P. sidoides* extract (Table 1). These results indicated that the increase in the *P. sidoides* extract dosage to some extent in diet enhances weight gains in common carp.

Discussion

The results of the present work have clearly demonstrated that *Pelargonium sidoides* extract-based diet affect basic haematological profile of common carp. The therapeutic use of *P. sidoides* extract in traditional medicine for the cure of infectious respiratory diseases and its present utilization in modern phytotherapy in Europe prompted our studies on the usage of *P. sidoides* extract as enhancing immune system efficiency in fish culture. This is a preliminary report to our knowledge regarding the potential of *Pelargonium sidoides* extract as antistress efficiency (based on the Hb content) in carp culture.

The haematological indices present a useful index, reflecting such culture conditions as the effects of dietary treatments on fish well-being, stress responses or as a diagnostic characteristic for the distinction of some infectious diseases (Hlavova 1993). Throughout the present study, the Hb level were significantly increased by *P. sidoides* extract (5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet). It could be concluded that insertion of *P. sidoides* extract had positive effects on such haemopoietic tissue as head kidney of common carp which may have helped the fish to adjust to such stressful conditions as either low oxygen availability or its being over dosed.

There are a number of specific haematological parameters recognized as valuable tools for monitoring fish health and physiological responses to environmental stress (Bahrami Babahydari *et al.* 2014; Banaee *et al.* 2017; Maneesh Kumar *et al.* 2018; Abdel-Tawwab and Monier 2018; Mohammadi *et al.* 2018), which suggested that icthyo-haematology is useful in the assessment of feed composition, nutritional status in relation to environmental conditions affecting fish. Among haematological parameters in fish, the white blood cells (WBC) afford protection against infectious agent caused by microbial and chemical factors (Harikrishnan *et al.* 2003). In present study, the WBC and Hb levels of 5 ml 100 g^{-1} *P. sidoides* extract-supplemented diet significantly increased ($P < 0.05$) from control and other groups. Ji *et al.* (2007) used dietary medicinal herbs feed to improve growth and haematological parameters in red sea bream, and survival, specific growth rate and hemoglobin levels were higher in fish given herbal diets than fish given the control diet without herbs. Immunostimulating activities of herbs has been reported in other fish, such as red tilapia (*Oreochromis niloticus*) (Rawling *et al.* 2009), tilapia *Oreochromis aureus* (Yin *et al.* 2006) and common carp *Cyprinus carpio* (Harikrishnan *et al.* 2003). Bahrami Babahydari *et al.* (2014) also reported that feeding common carp with the diet enriched with Wood Betony, *Stachys lavandulifolia* extract could enhance growth rate, improve some hematological characteristics with no adverse effects on body composition.

Conclusion

The present study demonstrated that *P. sidoides* extract had a positive effect on common carp growth and hematological parameters. It may be concluded that inclusion of *P. sidoides* extract in the diet of common carp can act as an antistress (based on the Hb content) in the species to promote the aquaculture production without any contrary effects on the fish. However, more research is required with different aquaculture species and longer time scales to fully evaluate the value of including *P. sidoides* extract at an industrial farming level.

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Pelargonium sidoides ekstraktının sazanda *Cyprinus carpio* Linneaus, 1758 hematolojik profil üzerine etkileri

Öz

Bu çalışmada, *Pelargonium sidoides* ekstraktının sazanda (*Cyprinus carpio*) hematolojik profil üzerine etkileri ilk kez araştırıldı. Araştırmada sazan yavruları (ortalama 0.67±0.05 g canlı ağırlık) 90 gün süre ile *P. sidoides* ekstrakt (0, 1, 3 ve 5 ml 100 g^{-1}) destekli deneme diyetleri ile beslendi. Deneme sonunda; en yüksek hemoglobin konsantrasyonu 3 ml 100 g^{-1} *P. sidoides* ekstrakt destekli diyet (10.50±0.45 g l^{-1}) ve 5 ml 100 g^{-1} *P. sidoides* destekli diyet (11.02±0.20 g l^{-1}) ile beslenen grupta tespit edilmiştir. *P. sidoides* ekstrakt-destekli diyet grupları arasında, en yüksek beyaz kan hücre değerleri (28.98±1.62 G l^{-1}) ve ortalama korpüsküler hemoglobin konsantrasyonu, 5 ml 100 g^{-1} *P. sidoides* ekstrakt-grubunda çıkmıştır. Diğer kan parametreleri açısından deneme grupları arasında istatistiksel anlamda bir farklılık gözlemlenmemiştir ($P < 0.05$). Bu çalışma; *P. sidoides* ekstraktını kullanılarak hazırlanan diyetlerin, yavru sazanların hematolojik profilini etkilediği sonucu çıkmıştır.

Anahtar kelimeler: Sazan, *Cyprinus carpio*, *Pelargonium sidoides*, hematolojik profil

References

Abdel-Tawwab, M., Monier, M. N. (2018) Stimulatory effect of dietary taurine on growth performance, digestive enzymes activity, antioxidant capacity, and tolerance of common carp, *Cyprinus carpio* L., fry to salinity stress. *Fish physiology and biochemistry* 44(2): 639-649.

Ahmadi, K., Banaee, M., Vosoghei, A.R., Mirvaghefei, A.R., Ataeimehr, B. (2012) Evaluation of the immunomodulatory effects of silymarin extract (*Silybum marianum*) on some immune parameters of rainbow trout,

Oncorhynchus mykiss (Actinopterygii: Salmoniformes: Salmonidae). *Acta Ichthyologica et Piscatoria* 42(2): 113-120.

Bahrami Babahydari, S., Dorafshan, S., Paykan Heyrati, P., Mahboobi Soofiani, N., Vahabi M. R. (2014) The Physiological changes, growth performance and whole body composition of common carp, *Cyprinus carpio* fed on diet containing wood betony, *Stachys lavandulifolia* extract. *Journal of Agriculture Science Technology* 16: 1565-1574.

Banaee, M., Soleimany, V., Haghi, B.N. (2017) Therapeutic effects of marshmallow (*Althaea officinalis* L.) extract on plasma biochemical parameters of common carp infected with *Aeromonas hydrophila*. *Veterinary Research Forum* 8(2): 145-153.

Dawood, M.A.O., Koshio, S. (2016) Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture* 454: 243-251.

FAO (2014) The State of World Fisheries and Aquaculture: Opportunities and challenges, Rome: FAO, 243 pp.

Ghadikolaei A.H., Kamali A., Soltani M., Sharifian M. (2017) Effects of *Zingiber officinale* powder on growth parameters, survival rate and biochemical composition of body in juvenile common carp (*Cyprinus carpio*). *Iranian Journal of Fisheries Sciences* 16(1): 67-85.

Haidvogl, M., Schuster, R., Heger, M. (1996) Acute bronchitisim kintesalter-multizenter- studie zur wirksamkeit und vertraglichkeit des phytotherapeutikums umckaloabo. *Z Phytotherapy* 7: 300-313.

Harikrishnan, R., Nisha Rani, M., Balasundaram, C. (2003) Hemotological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture* 221: 41-50.

Hlavova, V. (1993) References values of the haematological indices in grayling (*Thymallus thymallus linnaeus*). *Comparative Biochemistry and Physiology* 105: 525-532.

Ji, S.C., Takaoka, O., Jeong, G.S., Lee, S.W., Ishimaru, K., Seoka, M. (2007) Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream *Pagrus major*. *Fisheries Science* 73: 63-69.

Kayser, O., Kolodziej, H. (1998) Antibacterial activity of extracts and constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. *Planta Medica.* 63: 508-510.

Koch, E., Iber, A. (2003) Treatment of rats with the *Pelargonium sidoides* extract EPs® 7630 has no effect on blood coagulation parameters or on the pharmacokinetics o warfarin. *Phytomedicine* 14: 40-45.

Kolodziej, H., Kayser, O., Radtke, O., Kiderlen, A., Koch, E. (2003) Pharmacological profile of extracts of *Pelargonium sidoides* and their constituents. *Phytomedicine* 10(4): 18-24.

Kumar Bairwa, M., Kumar Jakhar, J., Satyanarayana, Y., Devivaraprasad Reddy, A. (2012) Animal and plant originated immunostimulants used in aquaculture. *Journal of Natural Products Plant Resources* 2(3): 397-400.

Kwon, M.G., Kim, Y.C., Shon, Y.C., Park, S.I. (1999) The dietary supplementing effects of kugija, *Lycium chinese*, on immune responses of nile tilapia, *Oreochromis niloticus* to *Edwardsiella tarda*. *Journal of Fish Pathology* 1: 73-81.

Lee, K.J., Dabrowski, K., Rinchard, J., Gomez, C., Leszek, G., Vilchez, C. (2004) Supplementation of maca (*Lepidium meyenii*) tuber meal in diets improves growth rate and survival of rainbow trout *Oncorhynchus mykiss* (Walbaum) alevins and juveniles. *Aquaculture Research* 35: 215-223.

Magi, E., Sahk, M. (2003) Use of herbal medicine principle in local conditions. *Agraarteadus* 14: 172-178.

Maneesh Kumar, D., Anita, Chauhan, R.S. (2018) Study of growth promoting and immunostimulatory effect of phytobiotic *Glycyrrhiza glabra* on fingerlings of *Cyprinus carpio* haematopterus. *Indian Journal of Geo Marine Sciences (IJMS)* 47(06): 1180-1184.

Mohammadi, M., Soltani, M., Siahpoosh, A., Shamsaie, M. (2018) Effects of dietary supplementation of date palm (*Phoenix dactylifera*) seed extract on body composition, lipid peroxidation and tissue quality of common carp (*Cyprinus carpio*) juveniles based on the total volatile nitrogen test. *Iranian Journal of Fisheries Sciences* 17(2): 394-402.

Norusis, M.J. (1993) SPSS for Windows Advanced statistics release 6.0. SPSS Inc., USA, 578 pp.

Pakravan, S., Hajimoradloo, A., Ghorbani, R. (2012) 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 Research* 43: 861-869.

Rawling, M.D., Merrifield, D.L., Davies, S.J. (2009) Preliminary assessment of dietary supplementation of Sangrovit® on red tilapia (*Oreochromis niloticus*) growth performance and health. *Aquaculture* 294: 118-122.

Turan, F., Gezer, A., Bircan Yıldırım, Y. (2011) Preliminary assessment of dietary *Pelargonium sidoides* extract on Common carp, *Cyprinus carpio* (L. 1758) growth performance. *The Israeli Journal of Aquaculture Bamidgeh.* 63(1): 537-542.

Turan, F., Gürlek, M., Yağlıoğlu, D. (2007) Dietary red clover (*Trifolium pratense*) on growth performance of common carp (*Cyprinus carpio*). *Journal of Animal and Veterinary Advances* 6(12): 1429-1433.

Turan, F., Mazlum, Y., Yıldırım, Y.B., Gezer, A. (2012) Use of dietary *Pelargonium sidoides* extract to improve growth and body composition of narrow-clawed crayfish *Astacus leptodactylus* Eschscholtz, 1823 juveniles. *Turkish Journal of Fisheries and Aquatic Sciences* 12(1-2): 233-238.

Yin, G., Jeney, G., Racz, T., Xu, P., Jun, X., Jeney, Z. (2006) Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*. *Aquaculture* 253: 39-47.