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# Use of Dietary *Pelargonium sidoides* Extract to Improve Growth and Body Composition of Narrow-Clawed Crayfish *Astacus leptodactylus* Eschscholtz, 1823 Juveniles

**Funda Turan[1,*], Yavuz Mazlum[1], Yasemin Bircan Yıldırım[1], Armağan Gezer[1]**

[1] Mustafa Kemal University, Faculty of Fisheries and Aquaculture 31200 Iskenderun, Hatay.

* Corresponding Author: Tel.: +90.326 6141866; Fax: +90.326 6141866;
E-mail: turanfunda@yahoo.com

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**Abstract**

The effects of the *Pelargonium sidoides* extract on survival, growth, and body composition of juvenile freshwater crayfish (*Astacus leptodactylus)* were investigated. Newly hatched third instars of *A. leptodactylus* were fed with experimental diets prepared by using supplementation of *P. sidoides* extract (0, 0.5, 1 and 2 ml 100 $g^{-1}$) for 105 days. Growth rate was increased significantly in crayfish fed with 2 ml *P. sidoides* extract 100 $g^{-1}$ diet in comparison to the control groups ($P<0.05$). Feed conversion ratio, protein efficiency ratio and apparent net protein utilization were also significantly improved in group fed diet with 2 ml 100 $g^{-1}$ *P. sidoides* extract than that with control and the other groups. After 105 days of rearing the survival rate was found highest in 2 ml *P. sidoides* extract 100 $g^{-1}$ diet supplemented group (80.0%). Protein content (16.5%) of the 2 ml 100 $g^{-1}$ *P. sidoides* extract-supplemented group was significantly higher than the control and other dietary groups ($P<0.05$). The lipid contents were decreased in *P. sidoides* extract-supplementation. These results indicated that the *P. sidoides* extract is useful to improve growth, feed utilization and survival rate in freshwater crayfish, juvenile diets.

*Keywords*: *Pelargonium sidoides*, crayfish, *Astacus leptodactylus*, growth performance.

**Yavru Kerevitlerin (*Astacus leptodactylus* Eschscholtz, 1823) Büyümesi ve Vücut Kompozisyonlarının Geliştirilmesi Üzerine *Pelargonium sidoides* Ekstraktını Kullanımı**

**Özet**

Bu çalışmada, yavru kerevitlerin (*Astacus leptodactylus* Eschscholtz, 1823) büyüme, yaşama oranı ve vücut kompozisyonları üzerine *Pelargonium sidoides* ekstraktının etkileri araştırıldı. Yeni çıkmış üçüncü aşamadaki *A. leptodactylus* yavruları 105 gün süre ile *P. sidoides* ekstrakt (0, 0.5, 1 ve 2 ml 100 $g^{-1}$) destekli deneme diyetleri ile beslendi. Deneme sonunda; 2 ml 100 $g^{-1}$ *P. sidoides* ekstraktı ile beslenen grupta kontrol grubuna göre büyüme oranı istatistiksel anlamda önemli derecede artış gösterdi. Benzer şekilde; Yem değerlendirme oranı, Protein etkinlik oranı ve Görünür net protein kullanım oranlarının diğer gruplara göre 2 ml 100 $g^{-1}$ *P. sidoides* extraktı ile beslenen grupta daha iyi olduğu tespit edildi. Deneme sonunda, en yüksek hayatta kalma oranı %80 ile 2 ml 100 $g^{-1}$ *P. sidoides* extraktı ile beslenen grupta bulundu. Protein içeriği yönünden en iyi grup, %16,5 ile 2 ml 100 $g^{-1}$ *P. sidoides* extraktı ile beslenen grup olduğu belirlendi. Yağ içeriği ise diyetlerdeki *P. sidoides* ekstraktının kullanımına bağlı olarak azalma gösterdi. Bu çalışma; *P. sidoides* ekstraktını kullanılarak hazırlanan diyetlerin, yavru kerevitlerde büyüme, yaşama oranı ve vücut kompozisyonları üzerine olumlu yönde etkili olduğu sonucunu göstermiştir.

*Anahtar Kelimeler*: *Pelargonium sidoides* kerevit, *Astacus leptodactylus*, büyüme.

## Introduction

The narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823, is one of the most important crayfish species in Europe due to its aquaculture potential and wide consumer demand. It has been widely introduced into many countries, e.g. Poland, Italy, Germany, England and France, where it escaped into the wild and established large populations in a number of water bodies (Skurdal and Taugbol, 2002). The only native crayfish species of Turkey, *A. leptodactylus* has a widespread distribution in lakes and ponds throughout the country (Harlıoğlu and Holdich, 2001; Skurdal and Taugbol, 2001; Mazlum, 2007; Harlioglu, 2009). At present it can be found in 30 countries, many of which it has been translocated to for stocking purposes (Souty-Grosset *et al*., 2006). Until 1984, freshwater crayfish had an

important role as an export product, but after 1986 crayfish production declined dramatically (from 5000 tones to 200 tones) in most Turkish lakes, because of the crayfish plague (*Aphanomyces astaci*) and through over fishing, pollution and agriculture irrigation (Bolat, 2001; Mazlum, 2007; Harlioglu, 2004). Since the market value of narrow-clawed crayfish in Europe is higher than the one of imported species, it is more profitable to develop the farming of the former (Holdich, 1993). Crayfish farming is also played a significant role in the active protection of native species for restocking programmes. (Carral *et al*., 2003; Smietana *et al*., 2004). Therefore, there is a need to demand for crayfish reproduction under controlled conditions.

Medicinal herbs are efficacious for growth, health management, and for the immune systems of land mammals and humans. They originate mainly from vegetables are popular medicines in eastern Asia and Africa. Such treatment creates synergistic effects on various biological functions and mechanisms (Wu *et al*., 2001; Park *et al*., 2001; Lee *et al*., 2003). One of the most important medicine herbs used in folk medicine by the Southern African native population is *Pelargonium sidoides* extract. The root of Umcka (*Pelargonium sidoides)* is used in traditional medicine to cure infectious respiratory diseases including tuberculosis (Haidvogl *et al*., 1996). *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 Biber, 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 amounts. These were identified as the prominent immunomodulatory principle for this herbal medicine (Kayser and Kolodziej, 1998). In addition, *P. sidoides* is rich in phytochemical, vitamins, minerals and amino acids that enhance the body's functioning and protects it against diseases (Kolodziej *et al*., 2003).

Some studies have been done in which herbs, as dietary additives, were fed to fish and shrimp. The focus of these studies includes their use as feeding attractants and their effects on growth, survival and immune system activity (Harada, 1991; Kwon *et al*., 1999; Lee *et al*., 2001; Jung *et al*., 2002; Kim *et al*., 2003; Immanuel *et al*., 2004; Lee *et al*., 2004; Ji *et al*., 2007). Medicinal herbs promote lipid metabolism that catabolizes body fatty acids as a main energy expenditure, resulting in efficient protein accumulation and growth performance (Immanuel *et al*., 2004). Also, Gezer (2009) has reported that *Pelargonium sidoides* extract -based diet improves growth and increase the level of protein in common carp. Although previous studies have mostly investigated on effects of herbal extracts on immunity and growth performance, there is a lack of data on the use of *P. sidoides* extract in diets for crayfish growth.

In the present study we intended to ascertain whether *P. sidoides* extract included in the diet enhance the growth performance and survival rate of the narrow-clawed crayfish, juvenile stage under laboratory condition.

## Materials and Methods

Forty-two ovigerous female *Astacus leptodactylus* were collected from experimental ponds at Eğirdir Fisheries Research Institute located in Eğirdir, Isparta, Turkey, on three occasions, all on 1 May 2007. After each collection, the crayfish were immediately packed in insulated live-fish shipping boxes and shipped by bus to Iskenderun, Turkey, where they were transported by car to the Aquaculture Research Facilities at Mustafa Kemal University where spawning took place, survival at arrival was 95.2%.

Prior to experimental use, the crayfish were kept in circular fiberglass tanks provided with shelter (e.g., onion sacks and pieces of polyvinylchloride pipe), and well-aerated water was used in the flow-through system. The animals were fed commercial carp diet to develop and shed eggs. Hatched larvae were reared until the third-instar stage (young-of-the-year, YOY). Total length (TL) of subsamples of the *A. leptodactylus* third instars was measured to the nearest millimeter, to estimate the size of the starting YOY crayfish.

Newly hatched third instars of *A. leptodactylus* with mean body weight 42.4±1 mg were fed with a commercial carp diet, and stocked into 60 L aquaria at a density of 10 crayfish per aquarium (50 crayfish/$m^2$) (Mazlum, 2007). Experiment was conducted in aquaria (n=12) with the dimensions of 0.8x0.4x0.25 m (length x width x height; area: 0.20 $m^2$). Each aquarium was supplied with continues aeration. A static system was used and 20% of the water in each aquarium was changed with 7 days interval. The bottom of each tank was provided with a PVC pipe and shelter.

Carp diets (Aquamak, Turkey: 28% protein, 12% lipid (on wet basis)) were used to prepare experimental diets. *Pelargonium sidoides* extract (UMCA®) was supplied by Dr. Willmar Schwabe GmbHandCo. (Ettlingen, Germany). (UMCA® contents; coumarins, gallic acid, polychemical ingredients). In the preparation of experimental diet, liquid *P. sidoides* extract were mixed with a solid carp diet in which water (450 mL kg-1) were added and pelletized through a food grinder with a 2 –mm diameter die plate. These pelleted diets were dried in the open air for 24 hours. The dry pellets were placed in covered plastic containers and stored in a refrigerator at +5°C (Lee *et al*., 2004). Three different dosages of *P. sidoides* extract (0.5, 1, 2 ml extract 100g-1diet) were used in the experiment. The control diet was also mixed with 450 ml water. The

extrusions were broken into small pieces and stored in freezer until feeding. Each of the three experimental diets was randomly assigned to triplicate groups. Feed was provided twice a day, at a quantity of 5% of their body weight, and uneaten food was removed before next feeding for 105 days (Mazlum *et al*., 2011). The amounts of feeds were adjusted according to the crayfish weight calculated for each sampling periods.

Water temperature and oxygen content were measured daily with a thermometer and a model 55 YSI oxygen meter (Yellow Springs Instruments Cy. Ohio), respectively. Water samples were collected for analysis per week: pH was determined with Accumet pH meter (Model 915, Fisher Scientific, Pennsylvania), ammonia nitrogen N-$NH_4$ (Nessler method) and nitrite nitrogen N-$NO_2$ (sulphanil method) were determined colorimetrically on spectrophotometer (UV-160 1 PC, Shimadzu visible). The average water temperature was 25±1.5 $^oC$, and the oxygen content of the water was 8.5±0.5. At pH 7.8±0.9, 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. The photoperiod was maintained on a 12-h light: 12-h dark schedule.

After 45, 75 and 105 days all the crayfish were collected and counted. The whole stock was individually weighed to the nearest 0.1 g. weight gain (Watanabe *et al*., 1990), feed conversion ratio (Steffens, 1989), specific growth rate (Clark *et al*., 1990), protein efficiency ratio (Steffens, 1989), apparent net protein utilization (Bender and Miller, 1953) and survival rate (Watanabe *et al.* 1990) were calculated at the end of the experiment. Then, subsample of ten crayfish were pooled and stored at – 20 $^oC$ for proximate analysis. Standard methods (AOAC, 1990) as described for the experimental diets were used to determine the initial and final whole body proximate composition.

In the experiment, the variables were tested for normality and homogeneity of variances using the UNIVARIATE procedure. The data were homogenous and showed normal distribution, and all data were subjected to a one-way analysis of variance (ANOVA) to determine if there is a difference in weight gain and body composition among treatments. Duncan test was used to compare the means of the treatments when differences occurred (Norusis, 1993).

## Results and Discussion

The effects of different concentrations of dietary *P. sidoides* extract on growth and survival of juvenile crayfish (*Astacus leptodactylus)* for 105 days are shown in Table 1. After 105 days of rearing the survival rate was highest in 2 ml *P. sidoides* extract 100$g^{-1}$ diet supplemented group (80.0%) and was lowest in control group (53.3%). Morever, there was no adverse influence of *Pelargonium sidoides* extract on the health status of the crayfish larvae in the present study. During the study, there were not abnornal observation on the behavior and general activities of treated cray fish.

Growth rate and Specific growth rate (SGR) were significantly increased in crayfish fed with *P. sidoides* extract-supplemented diets in comparison to the control groups ($P<0.05$). Among the *P. sidoides* extract-supplemented groups, the crayfish fed diet with 2 ml 100 $g^{-1}$ *P. sidoides* extract exhibited significantly higher than crayfish fed diets with 0.5 and 1 ml 100 $g^{-1}$ *P. sidoides* extract (Table 1). Feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were also significantly improved in group fed diet with 2 ml 100 $g^{-1}$ *P. sidoides* extract than that with control, 0.5 and 1 ml 100 $g^{-1}$ *P. sidoides* extract groups ($P<0.05$, Table 1).

The effects of different concentrations of dietary *P. sidoides* extract on the proximate composition of the whole-body juvenile freshwater crayfish (*Astacus leptodactylus)* for 105 days are shown in Table 2. Carcass moisture at the end the rearing trial did not change with dietary *P. sidoides* extract concentrations. Whole- body ash was significantly lower in 2 ml 100 $g^{-1}$ *P. sidoides* extract group than in

**Table 1.** The effects of different concentrations of dietary *P. sidoides* extract on growth and survival of juvenile freshwater crayfish (*Astacus leptodactylus)* for 105 days*

| Parameters | *P. sidoides* (ml 100$g^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 0.5 | 1 | 2 |
| Weight gain (g) | 1.27±0.08$^a$ | 1.41±0.04$^a$ | 1.42±0.05$^a$ | 1.88±0.11$^b$ |
| SGR | 3.23±0.10$^a$ | 3.37±0.05$^a$ | 3.42±0.06$^{ab}$ | 3.61±0.02$^b$ |
| FCR | 2.19±0.24$^b$ | 1.92±0.02$^b$ | 2.03±0.06$^b$ | 1.45±0.07$^a$ |
| PER | 1.33±0.14$^a$ | 1.49±0.01$^a$ | 1.40±0.04$^a$ | 1.97±0.10$^b$ |
| ANPU (%) | 19.15±1.24$^a$ | 20.43±0.48$^a$ | 22.11±1.30$^a$ | 32.26±2.51$^b$ |
| Survival rate (%) | 53.33±3.34$^a$ | 60.00±5.77$^a$ | 63.33±3.33$^a$ | 80.00±5.78$^b$ |

*Values (mean ± S.E. of triplicate) with different superscripts in each line indicate significant differences ($P<0.05$).

WG (Weight Gain) (g) = Final weight-Initial weight.

SGR (Specific Growth Rate) (%)= $[(\ln W^2-\ln W^1) \setminus (T^2-T^1)] \times 100$, where $W^1$, and $W^2$ are mean body weight at times when the first and second samples were taken ($T^1$ and $T^2$)

FCR (Food Conversion Ratio) = Feed consumption /weight gain

PER (Protein Efficiency Ratio) = Live body weight gained (g)/protein intake (g).

ANPU (Apparent Net Protein Utilization) (%)=[protein retained/unit of protein intake]x100.

**Table 2.** Proximate composition of the whole-body juvenile freshwater crayfish (*Astacus leptodactylus)* fed on diets containing different concentrations of dietary *P. sidoides* extract for 105 days*

| Parameters | | *P. sidoides* extract (ml 100g$^{-1}$) | | | |
|---|---|---|---|---|---|
| (%) | Initial | 0 | 0.5 | 1 | 2 |
| Moisture | 76.60±0.57 | 76.68±0.38$^{a}$ | 77.43±0.46$^{a}$ | 77.01±0.41$^{a}$ | 76.80±0.52$^{a}$ |
| Crude protein | 14.66±0.84 | 14.30±0.64$^{a}$ | 14.93±0.27$^{a}$ | 15.78±0.71$^{ab}$ | 16.51±0.39$^{b}$ |
| Crude lipid | 1.76±0.03 | 1.71±0.04$^{d}$ | 1.51±0.02$^{c}$ | 1.33±0.03$^{b}$ | 1.13±0.03$^{a}$ |
| Ash | 6.87±0.05 | 6.78±0.13$^{b}$ | 6.15±0.16$^{ab}$ | 6.57±0.31$^{b}$ | 5.45±0.25$^{a}$ |

*Values (mean ± S.E. of triplicate) with different superscripts in each line indicate significant differences (P<0.05).
Body composition data presented on a wet basis.

other dietary groups (Table 2). The protein contents of the whole body (16.51 %) of the 2 ml 100 g$^{-1}$ *P. sidoides* extract-supplemented group was significantly higher than protein contents of the control and other dietary groups (P<0.05). The lipid contents in the crayfish were dramatically decreased with increasing rate of *P. sidoides* extract-supplementation, and the control group had content (1.71%) (Table 2).

This was the first attempt to investigate potential of *Pelargonium sidoides* extract as a feed additive in crayfish culture. *Pelargonium sidoides* extract have been reported to increase antimicrobial activity and immunmodulatory effect in some terrestrial vertebrate animals (Koch and Biber, 2007; Bademkiran *et al*., 2009). However, no detailed information was found on the effects of *Pelargonium sidoides* extract on crayfish survival and growth. Only a study (Gezer, 2009) has reported that *Pelargonium sidoides* extract -based diet improves growth and survival in common carp. Therefore, the therapeutic use of *P. sidoides* extract prompted our studies on the usage of *P. sidoides* extract as dietary additives in crayfish culture. The present investigation revealed that *Pelargonium sidoides* extract -based diet improve growth and increase the level of protein in freshwater crayfish.

Also, there was no adverse influence of diet with *P. sidoides* extract on survival, and feed intake of crayfish in the present study and, FCRs were significantly improved in experimental treatments. This finding indicated that the *P. sidoides* extract (2 ml 100 g$^{-1}$) is a positive dietary additive to induce effective technical and economical propagations for cultured crayfish. Similarly, in abalone *Haliotis discus hannai* (Lee *et al*., 2001), olive flounder *Paralichthys olivaceus* (Jung *et al*., 2002), shrimp *Penaes indicus* (Immanuel *et al*., 2004), African catfish *Clarias gariepinus* (Turan and Akyurt, 2005), tilapia *Oreochromis aureus* (Turan, 2006), and common carp *Cyprinus carpio* (Turan *et al*., 2007) herbs in diets promoted growth and feed efficiency. Kim *et al*. (1998), suggested that unknown factors in various medicinal herbs led to favorable results in fish and shrimp trials. Sivaram *et al*. (2004) have been reported that medicinal herbs promote lipid metabolism, protein accumulation and growth performance. *P. sidoides* is rich in photochemical, vitamins, minerals and amino acids that enhance the body's functioning and protects it against diseases (Kolodziej *et al*., 2003), and the presence of these phytochemical in the *P. sidoides* extract may stimulate growth in crayfish. From a proximate composition point of view, *P. sidoides* increased the level of protein in crayfish. Interestingly, we found that the survival of crayfish can be improved by *P. sidoides* extract-supplementation. Therefore, we expect that this result will stimulate a series of studies on the utilization of *P. sidoides* in diets for crayfish. Also *Pelargonium sidoides* extract is sold in local pharmacies in Turkey for about 3 US $ per bottle (50 ml) for human medication. If it is supplied in bulk, it is expected that it would cost less.

In conclusion, the present findings suggested that disease and stress resistance.

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